WHO EQAP for influenza

SARINET AND REVELAC-I FOURTH ANNUAL MEETINGS
PUNTA CANA, DOMINICAN REPUBLIC, MAY 2017
TUESDAY MAY 23 - 25 2017

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WHO EQAP for influenza

- Aim and scope
- Performance of EQAP 1 to 15
  - General
  - AMRO
- EQAP 15
Global influenza virus surveillance has been conducted through the WHO Global Influenza Surveillance and Response System (GISRS) for over half a century.

The laboratory network comprises:
- 6 WHO Collaborating Centres (CCs),
- 4 Essential Regulatory Laboratories and
- 143 institutions in 113 WHO Member States recognized by WHO as National Influenza Centres (NICs).
- ad hoc groups set up to address specific emerging issues

Robust laboratory diagnostics and timely surveillance are essential for the early detection of:
- the continuous evolution of influenza viruses
- the pandemic potential of non-seasonal influenza viruses
Aim and Scope

● Aim
  – to monitor the quality and comparability of the performance of participating laboratories.
  – was initiated in 2007 after the influenza A(H5N1) outbreaks in Asia
    • assesses the ability of NICs to detect influenza A(H5) viruses, which pose a pandemic threat.

● Scope
  – EQAP has evolved over the years, the scope being extended to include;
    – seasonal influenza A,
    – influenza B and other non-seasonal influenza A zoonotic viruses
    – Phenotypic/genotypic neuraminidase inhibitor (NAI) susceptibility testing for influenza A(H1N1)pdm09 viruses included on an optional basis.(2016)

● Summaries of the performance of participating laboratories have been reported in the Weekly Epidemiological Record.
Coordination and Implementation

- Coordinated by the WHO’s Global Influenza Programme and implemented in collaboration with the:
  - H5 Reference Laboratory and National Influenza Centre at the Centre for Health Protection
  - Department of Health
  - Hong Kong Special Administrative Region
  - China

- Implemented annually
  - with support from WHO regional offices
Evolution of EQAP virus composition

Viruses included in EQA Panels
2007 to 2016
PAHO countries and NIC status - 2016
PAHO países y estado NIC - 2016

Countries Países (N=30)
- Argentina
- Barbados
- Bolivia
- Brazil
- Canada
- Chile
- Colombia
- Costa Rica
- Cuba
- Dominican Republic
- Ecuador
- El Salvador
- French Guiana
- Guatemala
- Guyana
- Honduras
- Jamaica
- Mexico
- Nicaragua
- Panama
- Paraguay
- Peru
- Suriname
- Trinidad and Tobago
- United States of America
- Uruguay
- Venezuela

Estado NIC /NIC status - Yes (N=29)
Estado NIC /NIC status- No (N=9)

Number of Laboratories Número de laboratorios (N=38)
Performance of laboratories
EQAP 1 to 15
Participation of AMRO laboratories in the WHO EQAP

No. Labs vs. % participation for each panel.

Panel 1 to 15 with corresponding bars for number of labs and line graph for % participation.

World Health Organization
SARinet and REVELAC-i Fourth Annual Meetings, Punta Cana, Dominican Republic, May 23 - 25 2017
AMRO Laboratories with 100% correct results
EQAP 1 to 15
AMRO Laboratories with incorrect H1pdm09 results
EQAP 1 to 15
AMRO Laboratories with incorrect H 3 EQAP 1 to 15

H3 incorrect

No. labs

Panel

Panel

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
AMRO Laboratories with incorrect B
EQAP 1 to 15

Flu B incorrect

No. Labs

EQA Panel
AMRO Laboratories with incorrect H5
EQAP 1 to 15

H5 incorrect

Panel

No. Labs

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

0 1 2 3 4 5 6 7 8

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World Health Organization

15
AMRO Laboratories with incorrect H7
EQAP 1 to 15
AMRO Laboratories with incorrect H9
EQAP 1 to 15

H9 incorrect

No. Labs

Panel

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Distribution of EQA panel-15

- NICs and other national influenza laboratories invited to participate before the panels were dispatched.

- Panel 15 was dispatched:
  - April 2016 and June 2016

- Shipments are at ambient temperature by courier service

- Required to report results within 4 weeks after the date of sample reception.
WHO EQAP for influenza
Panel 15

143 institutions in 113 WHO Member States

174 laboratories 138 countries

151 laboratories

132 returned results (87.4%)
Vacuum-dried inactivated influenza viruses

Viruses
- grown in Madin-Darby canine kidney (MDCK) cells
- Inactivated by triton X-100
- Pre-distribution homogeneity and stability testing is performed on 10 random samples
Composition of panel 15

- Panel 15 consisted of 10 coded samples different concentrations of influenza viruses
  - influenza A(H5N1) of genetic clade 2.3.2.1;
  - influenza A(H5N6) of genetic clade 2.3.4.4;
  - influenza A(H1N1) pdm09;
  - influenza A(H3N2);
  - influenza A(H9N2);
  - influenza B (Yamagata lineage) and
  - a sample that contained no virus.
  - Two influenza A(H1N1)pdm09 samples designated for phenotypic/genotypic testing of NAI susceptibility were included upon request.

- Participants were instructed to reconstitute each sample with PCR-grade water prior to testing.

- A questionnaire on the
  - laboratory methods and
  - gene targets used for the EQAP
The number of on time for analysis in panel 15 was as follows: 151 (86.8%).
Methods of detection

- Various PCR protocols and testing strategies:
  - >50% used protocols from CDC, USA
  - The use of different PCR protocols did not yield apparent differences in performance.
The number of laboratories participating in the EQAP has remained fairly stable since panel 8 in 2010.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Average correct %</th>
<th>Average correct %</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQAP 1 to 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All viruses</td>
<td>80.6</td>
<td>88.9</td>
</tr>
<tr>
<td>H5</td>
<td>89</td>
<td>93.4</td>
</tr>
<tr>
<td>A(H5N1) 2.3.2.1</td>
<td></td>
<td>97.7</td>
</tr>
<tr>
<td>A(H5N6) 2.3.4.4</td>
<td></td>
<td>97.7</td>
</tr>
</tbody>
</table>
H5 detection
EQAP 15

● The correct rate for influenza A(H5) samples correlated inversely with virus concentrations.

● Among the 14 incorrect results for influenza A(H5) samples;
  – 6 were reported for the sample with the lowest virus concentration and included;
  – (5 reports of influenza untypeable or negative results)
  – ? assay sensitivity, based on
    • 2 participants performing conventional PCR
    • 1 assay with mismatches in primer/probe sequences, and
    • 2 reporting generally high Ct values (>30) for all panel samples.
A(H9N2) in EQAP 15 = A(H7) in EQAP 14
  - correct rate of detection = 140/151 (92.7%)

The number of participants performing subtyping test
  - A(H9) (52/151, 34.4%) panel 15
  - A(H7) (136/153, 88.9%) in panel 14

Routine inclusion of influenza A detection assay in the testing algorithm is recommended to maintain laboratory capacity to detect emerging or less commonly circulating influenza A virus subtypes.

With the continuous circulation of influenza A(H9) participants are encouraged to consider regularly adopting and reviewing their subtyping assays.
The false positive rate

- dropped to 0.7% (1/151) in panel 15
- compared with 2.0% (3/153) in panel 14.
- suspected sample swapping ($n=3$)
- transcription error ($n=1$)
- highlighting the importance of good laboratory practice.
Among all the 47 participants in NAI susceptibility testing, the majority were from:

- EUR ($n=27$),
- WPR ($n=8$),
- AMR ($n=5$),
- AFR ($n=3$),
- EMR ($n=3$) and
- SEAR ($n=1$).
Participation in NAI susceptibility testing

EQAP 15

- 47/151 (31.1%) participants reported NAI results
- 43/47 returned results for genotypic testing for NAI01-2016
  - 42/43 reported (highly) reduced inhibition by oseltamivir
  - nucleotide change (C823T) in the NA gene (corresponding with H275Y amino acid substitution)
  - Six participants also reported (highly) reduced inhibition by peramivir.
- 28/47 returned results for phenotypic testing
- 24/47 participants performed both tests
- methods used:
  - allelic discrimination by real-time RT-PCR (25 participants [58.1%])
  - Sanger sequencing (15 [34.9%])
  - pyrosequencing (5 [11.6%])
  - massive parallel sequencing (1 [3.0%]).
All 28 participants reported correct phenotypic interpretations.

- The observed fold difference values of neuraminidase inhibition between the 2 samples were more than 100 for all participants.

- This is consistent with the WHO criteria where NAI01-2016 is associated with highly reduced inhibition by oseltamivir.

All 22 participants= 100% reported zanamivir correctly

- normal inhibition for both samples.
Genotypic neuraminidase inhibitor susceptibility testing

- Two influenza A(H1N1)pdm09 samples:
  - NAI01-2016 (with highly reduced neuraminidase inhibition by oseltamivir)
  - NAI02-2016 (wild type)

- 4743/47 returned results for genotypic testing for NAI01-2016
  - 42/43 reported (highly) reduced inhibition by oseltamivir
  - nucleotide change (C823T) in the NA gene (corresponding with H275Y amino acid substitution)
  - Six participants also reported (highly) reduced inhibition by peramivir.

- 42 for NAI02-2016
  - 41/42 (97.6%) reported wild-type influenza A(H1N1)pdm09
The participation pattern reflected development in global testing capacity

continuous EQAP support would be beneficial.
Acknowledgements

Dr Janice Lo and her team at the:

H5 Reference Laboratory and National Influenza Centre at the Centre for Health Protection
Department of Health
Hong Kong Special Administrative Region
China
# Tables
<table>
<thead>
<tr>
<th>Samples – Échantillons</th>
<th>Number of participants reporting – Nombre de laboratoires ayant notifié des résultats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A(H5) – Virus grippaux A(H5)</td>
<td>Other influenza A subtype – Autres sous-types grippaux A</td>
</tr>
<tr>
<td>V01-2016 /clade 2.3.2.1</td>
<td>1</td>
</tr>
<tr>
<td>V06-2016 /clade 2.3.4.4</td>
<td>1</td>
</tr>
<tr>
<td>V08-2016 /clade 2.3.2.1</td>
<td>1</td>
</tr>
<tr>
<td>V10-2016 /clade 2.3.4.4</td>
<td>0</td>
</tr>
<tr>
<td>Non-influenza A (H5) – Virus grippaux non A(H5)</td>
<td>A(H1)pdm09</td>
</tr>
<tr>
<td>V02-2016/ B Yamagata</td>
<td>0</td>
</tr>
<tr>
<td>V03-2016/ A(H3)</td>
<td>1</td>
</tr>
<tr>
<td>V04-2016/ A(H1)pdm09</td>
<td>NA – SO</td>
</tr>
<tr>
<td>V05-2016/ Negative</td>
<td>1</td>
</tr>
<tr>
<td>V07-2016/ A(H1)pdm09</td>
<td>NA – SO</td>
</tr>
<tr>
<td>V09-2016/ A(H9)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Only includes laboratories that have indicated performance of the specific subtyping assay. – Inclut uniquement les laboratoires ayant indiqué que l’essai spécifique de sous-typage a été réalisé.
NA: not applicable. – SO: sans objet.
Table 1b  Details of influenza A(H1N1)pdm09 samples for optional neuraminidase inhibitor susceptibility testing

<table>
<thead>
<tr>
<th>Sample No. – N° de l’échantillon</th>
<th>Amino acid substitution(^a) (Nucleotide change detected) – Substitution d’acides aminés(^a) (modification nucléotidique détectée)</th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
<th>No. of participants with correct results – Nbre de laboratoires obtenant des résultats corrects</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAI01-2016</td>
<td>H275Y (C823T) (Highly) reduced inhibition – Inhibition (fortement) réduite Normal inhibition – Inhibition normale</td>
<td>42</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>NAI02-2016</td>
<td>Wild type – Type sauvage Normal inhibition – Inhibition normale Normal inhibition – Inhibition normale</td>
<td>41</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Residue position in N1 neuraminidase numbering. – Position du résidu dans la numérotation N1 de la neuraminidase.
<table>
<thead>
<tr>
<th>Influenza viruses – Virus grippaux</th>
<th>Virus (clade)a – Virus (clade)a</th>
<th>Sample number – Numéro de l’échantillon</th>
<th>Copies/μl b – Copies/μl b</th>
<th>No. (%) of laboratories correctly identifying sample (n=151) – Nombre (%) de laboratoires ayant correctement identifié l’échantillon (n=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(H5N1)</td>
<td>A/Hong Kong/5923/2012 (2.3.2.1)</td>
<td>V01-2016</td>
<td>7.21</td>
<td>145 (96.9)</td>
</tr>
<tr>
<td>A(H5N1)</td>
<td>Same virus as V01-2016 – Même virus que V01-2016</td>
<td>V08-2016</td>
<td>1.28 x 10^2</td>
<td>150 (99.3)</td>
</tr>
<tr>
<td>A(H5N6)</td>
<td>A/Perugia/Falcon/Hong Kong/15-04955/2015 (2.3.4.4)</td>
<td>V06-2016</td>
<td>9.40</td>
<td>147 (97.4)</td>
</tr>
<tr>
<td>A(H5N6)</td>
<td>Same virus as V06-2016 – Même virus que V06-2016</td>
<td>V10-2016</td>
<td>7.49 x 10^1</td>
<td>148 (98.0)</td>
</tr>
<tr>
<td>A(H1N1)pdm09</td>
<td>A/California/7/2009-like virus – Analogue à A/California/7/2009</td>
<td>V04-2016</td>
<td>1.27 x 10^2</td>
<td>147 (97.4)</td>
</tr>
<tr>
<td>A(H1N1)pdm09</td>
<td>A/California/7/2009-like virus – Analogue à A/California/7/2009</td>
<td>V07-2016</td>
<td>6.93 x 10^2</td>
<td>151 (100.0)</td>
</tr>
<tr>
<td>A(H3N2)</td>
<td>A/Hong Kong/4801/2014-like virus – Analogue à A/Hong Kong/4801/2014</td>
<td>V03-2016</td>
<td>6.57</td>
<td>146 (96.7)</td>
</tr>
<tr>
<td>A(H9N2)</td>
<td>A/Hong Kong/309/2014</td>
<td>V09-2016</td>
<td>2.55 x 10^1</td>
<td>140 (92.7)</td>
</tr>
<tr>
<td>B</td>
<td>B/Phuket/3073/2013-like virus (Yamagata lineage) – Analogue à B/Phuket/3073/2013 (Ignée Yamagata)</td>
<td>V02-2016</td>
<td>1.92 x 10^2</td>
<td>148 (98.0)</td>
</tr>
<tr>
<td>Negative – Négatif</td>
<td>NA – SO</td>
<td>V05-2016</td>
<td>NA – SO</td>
<td>150 (99.3)</td>
</tr>
</tbody>
</table>

a The nomenclature of influenza A(H5) was based on the HA gene. For additional information, see [http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/](http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/) – La nomenclature des virus A(H5) est basée sur le gène HA. Pour en savoir plus, consulter [http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/](http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/) – [http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/2344/en/](http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/2344/en/)

b Measured by real-time RT-PCR after 5 days of storage of inactivated virus at 25°C. – Mesuré par PCR en temps réel après 5 jours de conservation du virus inactivé à 25°C. NA: not applicable. – SO: sans objet