CDC Real-Time RT-PCR Support Strategies and Updates

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Virus Surveillance and Diagnosis Branch
Influenza Division
Centers for Disease Control and Prevention

• The findings and conclusions in this presentation are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention

• National Center for Immunization and Respiratory Diseases
• Influenza Division
Overview

- Testing strategies & algorithms for surveillance and diagnostic testing
- CDC rRT-PCR assays for detection and characterization of influenza
- Support strategies for rRT-PCR testing
  - CLSIS – protocols/procedures
  - IRR - Reagent kits, control materials
- A(H1N1)pdm09 reactivity and assay update
Influenza Viruses in Humans - 2017

Type A

- Avian H5
- Avian H6
- Avian H7
- Avian H9
- Avian H10
- H3v
- H1v

Seasonal Strains

- H1
- H2
- H3

- 1918
- 1940
- 1957
- 1968
- 1977
- 1997
- 2003
- 2009
- 2017

Type B

- B/Yam
- B/Vic

Potential Pandemic strains

- Avian H6
- Avian H7
- Avian H9
- Avian H10
- H3v
- H1v

- 1918
- 1940
- 1957
- 1968
- 1977
- 1997
- 2003
- 2009
- 2017

*This diagram illustrates the evolution of influenza viruses in humans, including seasonal strains and potential pandemic strains.*
Routine Surveillance
No Known risk for avian influenza

Perform RT-PCR testing for seasonal human influenza A and influenza B viruses

- **InfA (+)**
  - Test for H3, pdm H1, pdmInfA
    - InfA (+)
    - H3 or pdm H1 (+)
      - Report Results

- **InfB (+)**
  - Test for YAM, VIC
    - InfB (+)
    - YAM or VIC (+)
      - Report Results

- **InfA unsubtypable – possible novel influenza**
  - Based on risk assessment, test for H5, H7 and/or other novel strains w/RT-PCR
  - InfA inconclusive samples with possible SOIV
  - Inconclusive samples with possible coinfection/LAIV

Report Results
CDC Influenza Virus Real-time RT-PCR Reagent Kits

Influenza A/B Typing Panel (InfA, Inf B, RP)

- Influenza A Subtyping Panel (InfA, H3, pdmH1, pdmInfA)
- Influenza B genotyping Panel (InfB, YAM, VIC, RP)

- Influenza A H5 (Asian lineage) Subtyping Panel (InfA, H5a, H5b, RP)
- Influenza A EuH7 Assay (EuH7)
**Update: CDC Influenza A/Subtyping Kit (Ver2)**

<table>
<thead>
<tr>
<th>CDC Human Influenza Real-Time RT-PCR Diagnostic Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kit</td>
</tr>
<tr>
<td>Influenza A Subtyping Kit (Ver2) FluIVD03-6</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

- Update pdmH1 assay to ensure reactivity (Clade 6B.1)
- Remove H1 assay
- Update positive control – SIPC
- Qualify ZEN quenched probes
### Reactivity of Updated pdmH1 assay - Inclusivity

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Conc.</th>
<th>InfA</th>
<th>pdmInfA</th>
<th>pdmH1 ver2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/California/04/09</td>
<td>$10^{2.9}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/California/07/09</td>
<td>$10^{3.5}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Colorado/14/2012</td>
<td>$10^{2.1}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Florida/27/2001</td>
<td>$10^{2.9}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Florida/62/2014</td>
<td>$10^{2.2}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Maryland/13/2012</td>
<td>$10^{2.0}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Minnesota/03/2011</td>
<td>$10^{2.9}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/North Carolina/4/2014</td>
<td>$10^{2.3}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Utah/13/2016</td>
<td>$10^{2.5}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
<tr>
<td>A/Washington/24/2012</td>
<td>$10^{2.5}$</td>
<td>(3/3)</td>
<td>(3/3)</td>
<td>(3/3)</td>
</tr>
</tbody>
</table>
## Interpretation of Real-time RT-PCR Results

<table>
<thead>
<tr>
<th>InfA</th>
<th>H3</th>
<th>pdm InfA</th>
<th>pdmH1</th>
<th>B</th>
<th>RP</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/−</td>
<td>A/H3</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/−</td>
<td>A/H1pdm</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+/−</td>
<td>B</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Influenza A - Unsubtypable</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Potential novel influenza</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Influenza A detected;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Presumptive positive for</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Influenza A(H3N2) variant virus</td>
</tr>
</tbody>
</table>
## Reactivity of CDC EuH7 Assay

<table>
<thead>
<tr>
<th>Strain Designation</th>
<th>sub-type</th>
<th>Lineage</th>
<th>Invitrogen</th>
<th>Quanta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>InfA</td>
<td>EuH7</td>
</tr>
<tr>
<td>A/ANHUI/1/2013</td>
<td>H7N9</td>
<td>Eu¹</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A/DUCK/VIETNAM/NCVD-197/2009</td>
<td>H7N3</td>
<td>Eu</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A/TURKEY/ITALY/5425/07</td>
<td>H7N3</td>
<td>Eu</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A/Shoveler/Egypt/00017-NAMRU3/2007</td>
<td>H7N3</td>
<td>Eu</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A/Mallard/Netherlands/12/2000</td>
<td>H7N3</td>
<td>Eu</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A/Chicken/Italy/5074/1999</td>
<td>H7N1</td>
<td>Eu</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A/MEXICO/7218/2012</td>
<td>H7N3</td>
<td>N.Am.²</td>
<td>+</td>
<td>Undet</td>
</tr>
</tbody>
</table>

Support for Public Health and Research Laboratories
Reagent ordering - IRR

International Reagent Resource (IRR), formerly known as Influenza Reagent Resource (IRR), was established by the Centers for Disease Control and Prevention (CDC).

Please register and login to view a complete list of available materials.

Click here to join Mailing List

www.internationalreagentresource.org
## CDC rRT-PCR Influenza Kit Distribution Globally

<table>
<thead>
<tr>
<th>CDC Influenza Virus Real-time RT-PCR Panel (RUO)</th>
<th>Catalogue #</th>
<th>Shipped 2014</th>
<th>Shipped 2015</th>
<th>Shipped 2016</th>
<th>Shipped 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A/B Typing Panel</td>
<td>FluRUO-01</td>
<td>261</td>
<td>209</td>
<td>276</td>
<td>77</td>
</tr>
<tr>
<td>Influenza A (H1/H3/H1 pdm09) Subtyping Panel</td>
<td>FluRUO-04</td>
<td>139</td>
<td>146</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>Influenza A (H3/H1pdm09) Subtyping Panel (VER 2)</td>
<td>FluRUO-09</td>
<td></td>
<td></td>
<td>127</td>
<td>58</td>
</tr>
<tr>
<td>Influenza B Lineage Genotyping Panel</td>
<td>FluRUO-05</td>
<td>101</td>
<td>111</td>
<td>140</td>
<td>43</td>
</tr>
<tr>
<td>Influenza A(H5) (Asian Lineage) Subtyping Panel (VER 3)</td>
<td>FluRUO-06, FluRUO-08</td>
<td>80</td>
<td>83</td>
<td>121</td>
<td>21</td>
</tr>
<tr>
<td>Influenza A/H7 (Eurasian Lineage) Assay</td>
<td>FluRUO-07</td>
<td>58</td>
<td>59</td>
<td>86</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>639</td>
<td>608</td>
<td>836</td>
<td>219</td>
</tr>
</tbody>
</table>

as of 4th April 2017 in 117 countries
Countries receiving CDC rRT-PCR reagents

117 countries
## CDC rRT-PCR Influenza Kit Distribution to PAHO Countries

<table>
<thead>
<tr>
<th>CDC Influenza Virus Real-time RT-PCR Panel (RUO)</th>
<th>Catalogue #</th>
<th>Shipped 2014</th>
<th>Shipped 2015</th>
<th>Shipped 2016</th>
<th>Shipped 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A/B Typing Panel</td>
<td>FluRUO-01</td>
<td>33</td>
<td>38</td>
<td>41</td>
<td>21</td>
</tr>
<tr>
<td>Influenza A (H1/H3/H1 pdm09) Subtyping Panel</td>
<td>FluRUO-04</td>
<td>26</td>
<td>22</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Influenza A (H3/H1pdm09) Subtyping Panel (VER 2)</td>
<td>FluRUO-09</td>
<td></td>
<td></td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Influenza B Lineage Genotyping Panel</td>
<td>FluRUO-05</td>
<td>26</td>
<td>16</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>Influenza A(H5) (Asian Lineage) Subtyping Panel (VER 3)</td>
<td>FluRUO-06, FluRUO-08</td>
<td>10</td>
<td>9</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Influenza A/H7 (Eurasian Lineage) Assay</td>
<td>FluRUO-07</td>
<td>10</td>
<td>9</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>105</td>
<td>94</td>
<td>134</td>
<td>51</td>
</tr>
</tbody>
</table>

As of 17 May 2017
27 laboratories in 22 countries
PAHO countries receiving reagents from IRR - 2017

27 laboratories in 22 countries
CDC Sharepoint Site for Laboratory Support for Influenza Surveillance

- Provide public health and research laboratories access to multiple assays, procedures and methods depending on need (equipment, chemistry, etc.)
- Allow for coordinated communication with registered laboratories
- Provide timely notification of assay updates

www.cdc.gov/flu/clsis
Countries registered on CLSIS

90 countries
PAHO countries registered on CLSIS - 2017

46 registrants
19 countries
CDC Sharepoint Site for Laboratory Support for Influenza Surveillance

- Real-time rRT-PCR laboratory protocols for influenza
  - English, Spanish, Portuguese
- RNA extraction procedures (manual & automated)
- Package inserts for kits and controls
- Enzyme options
- Real-time PCR equipment options
- Primer/probe sequence information
  - Manufacturing options for probe chemistry (ZEN, BHQ)

www.cdc.gov/flu/clsis
CDC Sharepoint Site for Laboratory Support for Influenza Surveillance

UPDATES:

- Updated rRT-PCR procedures
  - Assay specific protocols for FluA subtyping and FluB genotyping.
- Package insert for updated FluA subtyping kit (Ver2)
- Assay sequence updates to pdmH1 primers/probe
- FluB YAM/VIC genotyping primer/probe sequences

www.cdc.gov/flu/clsis
CDC Sharepoint Site for Laboratory Support for Influenza Surveillance

PENDING UPDATES:

• Real-time PCR equipment procedures
  • Qiagen Rotorgene Assay Manager protocol
• Automated Nucleic Acid isolation procedures
  • Qiagen EZ1 Advanced XL
  • Roche MP96
• FluB YAM/VIC genotyping primer/probe sequences

www.cdc.gov/flu/clsis
CDC Sharepoint Site for Laboratory Support for Influenza Surveillance

ONGOING ASSAY DEVELOPMENT:

- rRT-PCR assays
  - N. Am. H7
  - N. Am H5
  - FluB genotyping – possible update
- Diagnostic sequencing
  - Targeted Sanger sequencing for genotype identification
    - H1pdm09 vs H1v genotypes
    - Human H3 vs H3v genotypes
  - B VIC deletion mutation variant
PAHO countries receiving support from CDC - 2017

<table>
<thead>
<tr>
<th>Support provided</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received IRR reagents</td>
<td>22</td>
</tr>
<tr>
<td>Registered on CLSIS (protocols)</td>
<td>19 (46 users)</td>
</tr>
<tr>
<td>Countries that submitted samples to CDC since 2014</td>
<td>38</td>
</tr>
<tr>
<td>Total Countries in Region</td>
<td>46</td>
</tr>
</tbody>
</table>
Qualification of Real-time PCR Dx Instruments and Nucleic Acid Extraction Platforms
Real-time PCR Platforms

- LifeTechnologies/ABI 7300~7500Fast
- LifeTechnologies/ABI Step One
- LifeTechnologies – ViIA7/QuantStudio Dx
- Qiagen Corbett Rotor-Gene
- Stratagene/Agilent Mx3000p/3005p
- BioRad CFX
- BioRad iCycler iQ4/iQ5
- BioRad MiniOpticon
- 3M Integrated Cycler
- Roche LC 2.0
- Roche LC 96
- Roche LC 480
- Roche LC Nano
Real-time PCR Platforms

Thermo
- 7500Fast/FastDx
- Step One
- QuantStudio 3/5
- QuantStudio

Qiagen
- Rotor-Gene

BioRad
- CFX

Agilent
- Mx3005p
- Aria
- Integrated Cycler

3M

Roche
- LC 480
- LC 96
- LC Nano
FDA cleared Real-time PCR Platforms Under Evaluation for Dx Qualification

Thermo – ABI QuantStudio Dx (QSDX)
- 96 well
- Assay set up locked
- Results analysis and reporting locked

Qiagen RotorGene Dx – (QMDX)
- 72 well
- Assay set up locked
- Results analysis and reporting locked
Automated Nucleic Acid Extraction

Roche
- Magnapure Compact
- Magnapure LC

Qiagen
- Magnapure

Kingfisher
- Magnapure

BioMerieux
- EZ1 Advanced XL
- Qiacube HT
- Qiasymphony
- EasyMag

Qiagen
- Qiacube
- EZ1 Advanced XL
- Qiacube HT
- Qiasymphony
Qiagen Extraction Platforms

Qiacube:
Purification of NA from up to 12 samples; spin-column format

EZ1 Advanced XL:
Purification of nucleic acids from 1-6 or 1-14 samples

QIAextractor:
8-96 samples per run.

QIASymphony:
Processing 1-96 samples - in batches of 24
“kingfisher”

Magnetic bead extraction system
Flexible format allows for multiple chemistry options
Chemistry setup necessary
Pre/post sample processing necessary
Various platform throughputs – 1-15; 24, 96

Multiple vendors – Fisher Scientific, Ambion MagMax, Promega, etc.
Diagnostic Extraction Platform Under Evaluation

Qiagen EZ1 Advanced XL

- 1-14 samples
- 20-40 minutes
- Reagents pre-packaged – no set up
- Tube-to-tube extraction – no post extraction processing
- GMP manufacturing for Dx testing

Roche Magnapure 96

- 8-96 samples
- Reagents pre-packaged – no set up
- Plate-to-plate extraction – pre/post extraction processing
- GMP manufacturing for Dx testing
Extraction Platforms Under Consideration

Qiagen
Qiasymphony

KingFisher
Diagnostics Development Team

- Lakshmi Malapati
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Kellie White (Quality Assurance)

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Sarah Schildecker

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Kanwar Bedi
Dennis Bagarozzi

No Man is an island
Thank You