New pandemic virus detection by PCR with fluorescent detection AmpliSens® Influenza A/H1-swine

The 2009 flu pandemic is a global outbreak caused by a new strain of influenza A virus subtype H1N1, referred to as the “novel H1N1” strain originally identified in April 2009, and commonly called “Swine flu”. In June 2009, World Health Organization declared that new influenza strain of swine-origin H1N1 is responsible for 2009 flu pandemic. Molecular diagnostic based on real time PCR is currently a method of choice for detection of novel pandemic Influenza virus such as strain A/California/04/2009 according to WHO and CDC recommendations.

AmpliSens® PCR Kits in Influenza A/H1N1 Testing

On suspicion “swine flu” virus presence patients specimens have to be tested by AmpliSens® PCR kits in two steps:

**Step №1**
- Influenza type A of B differentiation by AmpliSens® Influenza virus A/B PCR kit.
  - If influenza type A is negative that sample considered as “swine flu” negative.
  - If influenza type A is positive that sample have to be tested for presence of “swine flu”.

**Step №2**
- “Swine flu” virus detection by AmpliSens® Influenza virus A/H1-swine PCR kit.
  - If H1-swine is positive that samples considered as “SWINE FLU” POSITIVE.
  - If “swine flu” negative that for further consideration two optional steps can be done:
    - Exclusion of A/H5N1 virus by AmpliSens® Influenza virus A H5N1 PCR kit
    - Influenza virus strain typing by AmpliSens® Influenza virus A-type PCR kit

Workflow for Detection of New Pandemic Influenza A/H1N1

- AmpliSens® Influenza virus A/B PCR kit
  - Influenza type A positive
  - Influenza type B positive

- AmpliSens® Influenza virus A/H1-swine PCR kit
  - Influenza virus A/H1N1-swine positive
  - Influenza virus A/H1N1-swine negative

Workflow for Further Typing of Influenza A:

- AmpliSens® Influenza virus A-type PCR kit
  - Influenza H1
  - Influenza H3
  - Influenza N1
  - Influenza N2

- AmpliSens® Influenza virus A H5N1 PCR kit
  - Influenza H5
  - Influenza N1

Russia, 121021, Moscow
8/bldg1, Olsuf’evsky per.
Phone: +7 (495) 925-05-54
www.interlabservice.ru
AmpliSens® Influenza virus A/H1-swine

PCR kits for amplification and identification of cDNA of Influenza virus A/H1N1-swine

Benefits of AmpliSens® Influenza Virus A/H1-Swine PCR Kits

- Identification of gene HA type 1 swine (swine-H1N1) and no cross-reactions with seasonal type (Human-H1N1)
- Internal control sample allows to control all stages of analysis from extraction to amplification

Sample Types

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Recommended kits for extraction and reverse transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical material: nasopharyngeal swabs and smears, aspirates, sputum</td>
<td>AmpliSens® Mukolysin to liquefy mucus, AmpliSens® RIBO-sorb or RIBO-prep, Reverta-L</td>
</tr>
<tr>
<td>Autopsy material: lungs or trachea tissues</td>
<td>AmpliSens® RIBO-sorb, Reverta-L</td>
</tr>
</tbody>
</table>

AmpliSens® PCR Kits

<table>
<thead>
<tr>
<th>Kit name</th>
<th>Detection</th>
<th>Details &amp; Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpliSens® Influenza virus A/H1-swine-FRT</td>
<td></td>
<td>Adapted for Rotor-Gene 6000/3000 (Corbett Research), Rotor-Gene Q (QIAGEN).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mastermix pre-pipetted into 0.2 ml PCR-tubes and sealed with wax.</td>
</tr>
<tr>
<td>AmpliSens® Influenza virus A/H1-swine-FRT</td>
<td></td>
<td>Adapted for iQ5 iCycler (Bio-Rad).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mastermix pre-pipetted into 0.2 ml PCR-tubes and sealed with wax.</td>
</tr>
<tr>
<td>AmpliSens® Influenza virus A/H1-swine-FRT</td>
<td></td>
<td>Adapted for SmartCycler (Cepheid).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contains hot-start TaqF DNA polymerase.</td>
</tr>
<tr>
<td>AmpliSens® Influenza virus A/H1-swine-FEP</td>
<td></td>
<td>Mastermix pre-pipetted into 0.5 ml PCR-tubes and sealed with wax.</td>
</tr>
<tr>
<td>AmpliSens® Influenza virus A/H1-swine-FEP</td>
<td></td>
<td>Mastermix pre-pipetted into 0.2 ml PCR-tubes and sealed with wax.</td>
</tr>
</tbody>
</table>

All kits included amplification reagents only. Each kit for 50 tests.

Instruments for Fluorescent Detection:

- **FRT** — Fluorescence Real Time detection: Rotor-Gene 3000/6000 (Corbett Research), Rotor-Gene Q (QIAGEN), iCycler iQ5 (Bio-Rad), SmartCycler (Cepheid)
- **FEP** — Fluorescence detection with End Point analysis: ALA 1/4 (BioSan)

Analytical Data

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x10^3 copies/ml</td>
<td>No cross-reaction with other types of influenza virus A, influenza virus B, Respiratory-Syncytial virus, adenovirus, parainfluenza virus (subtypes 1-4).</td>
</tr>
</tbody>
</table>

All cases of respiratory infections caused by highly pathogenic influenza virus A/H1N1-swine in Russia, were confirmed with AmpliSens® PCR kits.