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PIV-33

Detection of oseltamivir-resistant influenza A(H1N1) viruses with H274Y mutation during 2007–2008 influenza season from central and eastern part of Turkey

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In the beginning of 2007–2008 Northern Hemisphere influenza season, the frequency of influenza A(H1N1) viruses bearing a previously defined oseltamivir resistance conferring amino acid change from Histidine to Tyrosine at position 274 (H274Y) in neuraminidase (NA) gene increased dramatically. The overall frequency of oseltamivir resistance in A(H1N1) strains from Europe was 25%, although it varied between countries, with Norway detecting the highest proportion (67%), and others, including Spain, as low as 2%. In order to detect such resistant viruses in Turkey, an RT-PCR assay was performed targeting amino acid position 274 in NA gene of H1N1 influenza strain to investigate the presence or absence of histidine to tyrosine mutation.
A total of 20 influenza A(H1N1) isolates were selected randomly from 73 influenza A (H1N1) positive specimens from different provinces which were detected by real-time PCR in Refik Saydam Public Health Agency National Influenza Center, Turkey during the 2007–2008 influenza season. The NA genes of influenza viruses were sequenced and resistance was inferred by comparison with published sequences and known resistant mutations. 2 of them (10%) had histidine (H) to tyrosine (Y) *change* at position 274 (275 in N1 numbering) of the NA gene which indicates resistance to oseltamivir. By NA gene sequencing, we monitor the presence of H274Y oseltamivir-resistant influenza A(H1N1) viruses in central and eastern part of Turkey Our results indicate that resistance to NAIs began to be detected in A/H1N1 isolates in Turkey in 2008.

PIV-34

A fast procedure for the detection of the new influenza virus A/H1N1 variant

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Background: In response to the large outbreaks of the new influenza virus A/H1N1 (A/H1N1v) isolated in April 2009 a fast molecular procedure was evaluated.
Aim: To assess a fast molecular procedure for the identification of the new influenza virus A/H1N1v.
Methods: The procedure consisted of two steps: 1) the detection of the influenza type A (Iv-A) and B (Iv-B) viruses by a commercial real-time PCR (Nanogen, I and Arrow, I); 2) the identification of the swine-flu type A (matrix-gene) and the new influenza A/H1N1v (HA-gene) according to the WHO-CDC protocol (April, 2009) by a one-step real-time PCR system (AB-Ambion, US). 111 nasal or nasopharyngeal specimens from 111 patients were analyzed. Nucleic-acids extraction was performed with the NucliSENS-easyMAG (Biomerieux, F). Positive A/H1N1v samples were grown in MDCK cell cultures and sequenced (HA-segment, 1701bp).
Results: We identified 16 (14.4%) A/H1N1v, 5 (4.5%) seasonal Iv-A (H3N2) and 1 (1%) Iv-B. Five A/H1N1v were sequenced (HA-segment) and clustered with the reference GenBank A/H1N1v sequences. The total turn-around-time for positive and negative samples was 6.5hrs and 3hrs, respectively. Moreover 20 samples resulted positive for other respiratory viruses: 10 Rhinovirus, 3 Parainfluenza-3, 2 Adenovirus, 2 h-Coronavirus 229E/NL63, 1 h-Metapneumovirus, 1 RSV and one samples was co-infected with a parainfluenzavirus-3 and Rhinovirus.
Conclusion: Our procedure identified and typed A/H1N1v in one working-day and can be successfully used for the new influenza A/H1N1v surveillance allowing a prompt management of A/H1N1v infected patients as well as of their contacts.

PIV-35

Evaluation of two newly developed QIAsymphony sp protocols for efficient isolation of influenza virus RNA from different respiratory samples

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Purpose: Influenza is one of the most severe respiratory infections. Detection of the Influenza Virus by realtime PCR is sensitive and fast. Here we report the suitability of the QIAsymphony for isolation of Influenza RNA from different sample materials. In addition, the fully automated QIAsymphony protocol is compared to a protocol with off-board lysis.
Methods: Respiratory samples from swine and humans were spiked with Influenza standard. Viral RNA was extracted either with a manual method (QIAamp Viral RNA), or with the QIAsymphony Virus/Bacteria Mini Kit. For off-board lysis, samples were incubated with or without shaking in a lysis mixture similar to the one used in the completely automated procedure. Lysates were transferred onto the QIAsymphony and a Bind-Wash-Elute protocol was run. Purified RNA was analyzed using the artus Influenza RT PCR-Kit.
Results: All respiratory samples were detected positive with both extraction methods. In general, the QIAsymphony showed better performance compared to the manual method by an average over all samples delta Ct of −1.83.
No differences were observed between the fully automated protocol and the off-board lysis protocol using the QIAsymphony. Shaking during off-board lysis did not affect the performance.
Conclusion: The QIAsymphony Virus/Bacteria Kit in combination with the artus Influenza RT-PCR Kit is suitable for sensitive analysis of Influenza from different respiratory sample types.
The artus Influenza RT-PCR Kit is intended For Research Use Only. Not for use in diagnostics procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

PIV-36

Performance of the Qiagen Resplex II ver. 2.0 & ver. 3.0 multiplex assays for the detection of (H1N1V) pandemic influenza A in a London teaching hospital

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In April 2009, a new variant of H1N1, 'swine flu' or pandemic influenza A (H1N1v) emerged. Here we assess the ability of Resplex II ver 2.0 & 3.0, to detect the new flu variant, when compared with RT-PCR. Qiagen Respex II Ver.2.0 detects multiple viral pathogens, (RSVA&B, Influenza A&B, Rhinovirus, Coxsackie/Echo, Human Metapneumo, Adenovirus B/E, Coronavirus, Paraflu 1–4 and Bocavirus) using QIAplex and xMAP technologies. Ver.3.0 is an adapted product additionally detecting 'H1-M' (H1N1v).
Over a 4 week period from mid-June 09 we tested 393 in-patients for H1N1v and detected 73 positives by H1 RT-PCR adapted to include an MS2 internal control (HPAVSOP 29, Issue 2, May 2009). 206 patients were tested by both Resplex II ver2.0 and by RT-PCR. 25 selected H1N1v RT-PCR positive patients were additionally tested by Resplex II ver.3.0. More patient data is anticipated by Sept 09.
Resplex II ver.2.0 detected 13 of 37 confirmed H1N1v. (sensitivity 60.7%). 167 patients were negative by both methods. Resplex II ver. 3.0 detected 18 of 25 confirmed H1N1v. (sensitivity 80.0%). Only positive patients were tested by ver.3.0.
Resplex II delivers broad viral pathogenic coverage but as with all multiplex assays sensitivity can be an issue. We found that the Xtag multiplex is much less sensitive than H1 RT-PCR assay for H1N1v. Our conclusion is that Resplex II ver 2.0 & 3.0 are unsuitable for detecting low viral load H1N1v in its current form and should be supplemented with an additional assay in service to avoid false negatives.

PIV-37

Do viral respiratory co-pathogens contribute to morbidity & mortality in an outbreak of parainfluenza type 3 in haematology-oncology patients?

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Human Parainfluenza Type 3 (HPIV-3) is implicated in community and nosocomial outbreaks among haemoncology patients. From April to November 2008, 20 adult patients, including 18 human stem cell transplant recipients acquired HPIV-3 infection, diagnosed by direct immunofluorescence assay (DFA) on respiratory samples. Nine patients died, all of whom had significant