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for development of rapid tests for influenza as well as assay for rapid subtyping of these viruses. We have developed subtyping assays allowing for the detection and characterization of the present H1N1 and H3N2 viruses in one real-time PCR reaction using a four color taqman probe detection system. In another assay the H5, N1 from birds and H7 influenza genes can be detected. The assays run on the same condition and can be performed in 2 hours and uses extracted RNA directly from clinical material. We have used these assay for detection of the influenza A in patient samples through the last 2 years. All influenza A samples detected positive for conserved sequences in the M gene could be subtyped with these assays. Furthermore the avian influenza A assay were evaluated on avian samples with different hemagglutinin and neuraminidase subtypes. The avian assay was specific for H5 and H7 and for avian N1 – this assay detects both clade 1 and 2 of the present H5 viruses.

These use of these influenza A subtyping assays allows for the detection of present circulating strains as well as present circulating avian H5 and H7 strain and detection and subtyping can be done in few hours.

P18 Multiplex real time PCRs for respiratory viral infections

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Viral respiratory tract infections are some of the most common infection in all age groups. The etiological diagnosis can be difficult as more than 200 viruses virtually give the same symptoms. We developed 3 four-color multiplex PCR assays running on same conditions. The assays were able to detect and discriminate between influenza A and B, human metapneumovirus, RSV, parainfluenza 1, 2 and 3, coronavirus OC43, 229E and NL-63 as well as rhino- and adenoviruses. The assays were used on more than 2000 samples submitted to the department of virology in a 1.5 year period.

We found the expected distribution of viruses throughout the year and found that many viruses are present in the community especially during the wintertime where RSV, metapneumovirus, rhinoviruses and influenza were most prevalent. Many children had infections with more than one virus at a time. The assays were performed with a turn-around time of less than a working day and frozen ready-to-go mastermix containing all reagents could be stored. The use of such assays results in sensitive and rapid diagnosis of most viral respiratory tract infections.

P19 Life threatening human metapneumovirus infections in the West of Scotland

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Background: Human metapneumovirus (hMPV) is a recently discovered respiratory virus shown to cause lower and upper respiratory tract infections in persons of all age groups.

Aim: To assess the prevalence and severity of human metapneumovirus infections in the West of Scotland.

Methods: In the two winter seasons from August 2004 to April 2006, 9274 respiratory samples were screened for hMPV using real time RT-PCR, which is the largest survey to date.

Results: hMPV was detected in 206(2.2%) samples, with a detection rate of 3.89% in <1 yr of age, 2.19% in 1–4 yrs, 0.7% in 5–17 yrs, 0.44% in 18–44 yrs, 1.1% in 45–64 yrs and 0.61% in >65 yrs age groups. From a cohort of 5500 respiratory samples from adult patients (>18 yrs) with respiratory symptoms, 33 (0.6%) samples tested positive for hMPV and within this group 8 (24%) persons had severe lower respiratory tract infection, 4 of whom had an underlying immuno-suppressive disorder and needed ventilation support in ITU. Two of them died: in one, we were able to demonstrate hMPV in the autopsied lung sample, both by RT-PCR and immuno-staining. The rapidity of the worsening of the respiratory infection was a common observation in all 4. No other respiratory pathogens were identified in them, except for a rhinovirus in one.

Discussion: This study emphasises the importance of including hMPV in the respiratory screening panel, and further highlights

the value of rapid diagnostic techniques such as real time RT-PCR, in the diagnosis of respiratory infections, especially in the immunosuppressed.

P20 Influenza A detection, typing and assessment of antiviral resistance

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Background and Aim: Rapid detection and analysis of influenza A viruses is important for patient and outbreak management. The aim of this study was to evaluate nucleic acid amplification tests (NATs) for detection, sub-typing and sequence analysis of influenza A without prior culture of specimens.

Methods: Respiratory specimens, isolates and proficiency panel samples were analysed for this study. Nucleic acid preparation was automated using the easyMAG[®] extractor (bioMérieux). Real time NATs (NASBA and PCR) were utilized for detection of influenza A. Influenza A subtyping was undertaken using real-time RT-PCR assays to determine haemagglutinin (H1, H3, H5, H7) and neuraminidase (N1, N2) types. Sequencing and analysis of the HA1 and M2 genes utilized cycle-sequencing (Applied Biosystems) and/or pyrosequencing chemistries (Biotage).

Results: Sensitivity and specificity of NATs for detection of circulating influenza A viruses was excellent (>8000 specimens). The feasibility of direct amplification, typing and sequence analysis of influenza A from clinical specimens was confirmed. HA1 gene sequence was useful to assess relatedness of strains and any drift from the vaccine strain. Since 2004, there has been a significant increase in genotypic markers conferring amantadine resistance in circulating influenza A strains in Alberta (91% resistant in 2006).

Discussion and Conclusions: Molecular amplification assays are a suitable and potentially more sensitive alternative to conventional methods for diagnosis of influenza A. Direct analysis of respiratory specimens (without prior culture) is feasible and real-time provision of influenza A subtyping, strain drift and antiviral resistance data will have a positive impact on outbreak management and pandemic preparedness.

P21 Nucleic acid amplification assays for investigation of respiratory viruses

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Background and Aim: Nucleic acid amplification tests (NATs) provide enhanced sensitivity for diagnosis of respiratory viruses. The aim of this study was to assess the utility of NATs with a focus on detection of adenoviruses (ADV), human metapneumovirus (hMPV), human coronaviruses (HCoVs) and human bocavirus (HBoV).

Methods: Respiratory samples collected in 2005–2006 were utilized for this study (n > 8000). Nucleic acid was prepared using the easyMAG[®] extractor (bioMérieux). Our routine diagnostic algorithm, which includes NATs for identification of influenza (IFV) A and B, parainfluenza (PIV) 1–4, respiratory syncytial virus (RSV), ADV and hMPV, was applied to all samples. A subset of samples was analyzed for detectable HCoV (229E, OC43, NL63 and HKU1) and HBoV sequences using newly designed and validated real-time PCR assays (hydrolysis probe chemistry using the ABI 7500).

Results: As anticipated, NATs provided enhanced sensitivity for detection of IFV, PIV and RSV infections. Six and fourteen percent of respiratory samples contained detectable ADV DNA or hMPV RNA, respectively. hMPV was associated with a seasonal winter peak and outbreaks in the elderly. Co-infections with other respiratory viruses were relatively common in all patient groups (17% of hMPV positives). 5% of the tested respiratory samples were positive for HBoV. HCoVs were identified in 3% of samples from adults and 5% of samples from children.

Discussion and Conclusions: NATs for respiratory viruses were able to resolve respiratory infections of previously unknown etiology. Expansion of routine NAT panels to include recently identified respiratory viruses is recommended.