## P9  Identification of respiratory viruses detected during three consecutive winter seasons

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In order to identify the different viral agents more frequently involved in mild and severe respiratory tract infections, 614 symptomatic patients, 351 attending the pediatric and 263 the hematology units of the General Hospital of Bolzano, Italy, were investigated during three consecutive seasons, from October 2003 through April 2006. Bronchoalveolar lavage, bronchial aspirate, nasopharyngeal secretion and nasopharyngeal lavage specimens were examined by direct fluorescent antibody assay and, where possible, shell vial culture technique for the presence of influenza virus A/B (FluA/B), Para influenza virus 1,2,3 (PIV1,2,3), Adenoviruses (Adeno), Respiratory syncytial virus (RSV) and, for the last season, human-Metapneumovirus (hMPV).

During the entire study period we identified 185 different viral infections (30%): 147 patients were infected by RSV (79%), followed by 13 FluA (7%), 9 hMPV (5%), 9 PIV3 (5%), 3 Adeno (2%), 2 PIV2 (1%), 1 FluB (0.5%) and 1 PIV1 (0.5%).

Presence of the virus was confirmed in 7 out of the 9 hMPV positive samples by RT-PCR. Sequencing of the amplified hMPV-NP-gene-fragments indicated a different genotype compared to the isolates obtained in the nearby North-Tyrolean region earlier (Larcher et al., JHLT, 2005).

The majority of the viruses have been detected in the pediatric patients, however the PIV1,2,3 seem to be more frequent in the hematological patients. While RSV remains the prevalent virus in the pediatric subgroup for every examined season, this was not repeatedly observed in hematological patients. Moreover, while for the 2004–2005 season no PIV have been identified, we did not detect presence of FluA/B last season.

## P10  Mosaic structure of human coronavirus NL63, one thousand years of evolution

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**Background and Aims:** Before the SARS-CoV outbreak only 2 human coronaviruses (HCoV) were known: HCoV-OC43 and HCoV-229E. In 2004 a fourth human coronavirus (HCoV-NL63) was discovered in The Netherlands. Little is known about the heterogeneity of HCoV-NL63 isolates, and its evolutionary characteristics.

**Methods:** We determined the complete genome sequence of two HCoV-NL63 clinical isolates, designated 057 and 496. Furthermore, of 21 additional NL63-isolates various genome fragments were analyzed to confirm phylogenetic clustering.

**Results and Conclusions:** The genomes of the clinical isolates of HCoV-NL63 are 27538 and 27550 nucleotides long, and share the same genome organization. We compared the new isolates with the prototype strains Amsterdam 1 and NL and observed 99% sequence similarity between isolates, with 497 heterogeneous positions. Clustering of these variable sites suggests the presence of two hypervariable regions in the 1a and S genes. The 1b and N genes were most conserved. Phylogenetic analysis indicates the presence of HCoV-NL63 variants with distinct genetic markers. Bootscan analysis revealed that the genomes have a mosaic structure with multiple recombination sites. Employing three different algorithms we assessed the mutation rate for the S gene of group Ib coronaviruses to be $\sim 3 \text{times}; 10^{-4}$ mutations per site per year and the split of HCoV-NL63 and its closest relative, HCoV-229E, was dated back to the eleventh century.

## P11  Diagnosis of human metapneumovirus by immunofluorescence – the Newcastle experience

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**Background and Aims:** Human metapneumovirus (hMPV) was discovered in 2001. hMPV has a worldwide distribution and has a spectrum of respiratory illness in children similar to Respiratory Syncytial Virus (RSV). The aim of the study was to investigate the prevalence of hMPV infection in hospitalized patients in north east England during April 2005 to April 2006 using immunoflourescence (IF).

**Methods:** A polyclonal antiserum to hMPV was prepared and used in an indirect IF assay. The reagent was validated against an in house hMPV RT PCR (F and N gene targets). IF was also performed using a pool of monoclonal antibodies and compared with the polyclonal antiserum.

**Results:** Indirect IF using the polyclonal antiserum had a sensitivity of 89 percent and a sensitivity of 96 percent when validated against RT PCR. 857 samples were tested by both polyclonal and monoclonal antibody reagents with a concordance of 99.8 percent. 1860 respiratory samples were tested, of which 52 percent were from children aged less than 1 year. The mean overall positivity rate for hMPV was 5 percent. hMPV was the second most prevalent virus after RSV, with 92 and 343 positive samples respectively. The peak months of hMPV prevalence were November to March.

**Conclusions:** hMPV is widely prevalent in hospitalized patients in the north east of England and was the second most commonly diagnosed virus after RSV. Diagnosis of hMPV by indirect IF was sensitive, specific and practical. The monoclonal antibody pool and polyclonal antiserum were equally effective in diagnosing hMPV by IF.

## P12  Detection of human metapneumovirus in clinical specimens using a novel immunoassay

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**Background and Aims:** Human Metapneumovirus (hMPV) has been described as a causative agent for acute respiratory tract infections and is considered one of the main causes of hospitalisation for such infections in young children worldwide. There is currently a requirement for an antigen detection assay for respiratory screening. Here we describe an antigen detection EIA utilising a combination of monoclonal antibodies directed to major hMPV proteins.

**Methods:** The performance characteristics of the immunoassay system were evaluated using panels of PCR-confirmed hMPV-positive or -negative respiratory specimens from two separate clinical laboratories.

**Results:** The prototype assay results exhibited close correlation to PCR data. A high level of specificity was demonstrated with a panel of potentially cross-reactive, non-hMPV specimens. The newly developed immunoassay has been found to be reactive with hMPV subgroups 1 and 2 of genotypes A and B from viral culture.

**Conclusions:** This efficient, highly sensitive and specific assay will have useful implications for hMPV detection in respiratory specimens and/or in culture confirmation.