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**P22** Increased rates of respiratory viral (RV) infections using Flocked Swabs and UTM-RT

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**Background:** Detection of antigens, nucleic acids, and isolation of microbes depend on pre-analytical devices used for collection of specimens. Diagnostic sensitivity varies with the number of microbial targets released and protected in the transport system. Previously Flocked Swabs (FS) and universal transport medium at room temperature (UTM-RT) [Copan, Brescia, Italy] enhanced analytical sensitivity with PCR assays.

**Aims:** To determine whether RV positivity rates are different when nasopharyngeal swabs (NPS) are collected by FS into UTM-RT to diagnose RV using antigen, cell culture and PCR assays.

**Methods:** NPS (n=2883) collected with FS and UTM-RT, from 11/01/04 to 02/28/06 were compared to NPS collected with an M4-RT collection system from the same time frame the previous years. An aliquot of NPS was stored for PCR; then tested by DFA and shell vial culture or with 4 antigen tests for RSV, Flu A and B. NPS (n=261) were tested by PCR for Flu A/B and 375 for hMPV.

**Results:** The 2883 NPS DFA/culture had 416 (15%) Flu A, 150 (5%) Flu B, 502 (18%) RSV, 42 (2%) Para 1-3, 78 (3%) Adenovirus; 101/868 hMPV (15%). Antigen tests had 121 RSV, 33 Flu A, 37 Flu B and 100 negatives. The PCR detected 102/261 Flu, 66/375 hMPV. All rates were higher than the previous year.

**Conclusions:** NPS collected with FS in UTM-RT demonstrated higher positivity rates for each virus type with DFA/culture, rapid antigens and PCR. PCR assays for Flu A, B, and hMPV were more sensitive than DFA and culture.

**P23** Impact of PCR on our understanding of viral respiratory infections

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**Background and Aims:** To determine the influence of molecular versus culture and immunofluorescence (IF) tests on the epidemiology of common respiratory viruses i.e influenza A/B; respiratory syncytial virus; parainfluenza virus types 1-3, adenovirus, rhinoviruses, coronaviruses (CoV), human metapneumovirus (hMPV) and bocavirus.

**Methods:** A molecular respiratory virus panel was introduced in Belfast in July 2003. A retrospective comparison of this service compared to culture and immunofluorescence (IF) was undertaken. Three time periods were compared: (1) 1995-1997 when IF and virus culture were in use; (2) 2001-2003 when IF only was in use; (3) 2003-2006 when molecular tests only were in use.

**Results:** Molecular assays detected more virus infections than culture/IF and IF (55%, 40%, 26%) and also had higher respective levels of mixed infections (25%, 0.6%, 0%). Patterns of recovery suggested differences in the propensity of different virus genera to establish co-infections. CoV and hMPV infections were detected in 4% and 7% of specimens respectively and in a small subset of 220 patients, bocavirus was detected in 4 (1.8%) children, all with virus co-infections. Based on admission to ICU there was no evidence of increased severity of infection associated with co-infection.

**Discussion:** Issues like cost and result significance have challenged the widespread use of molecular assays. Also the diverse genera of respiratory viruses presents technical difficulties. However it is clear they provide a different data set to culture or IF. It is important that their clinical significance and the data emanating from their use becomes the subject of clinical focus.

**P24** Expression of C-C and CXC-chemokines by enterovirus-infected lower airway epithelial cells

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**Background:** Enteroviruses have been recently identified as major causative agents of acute bronchiolitis in children. In the present time, the inflammatory processes secondary to EV airway epithelial cell infection remains totally unknown.

**Aims:** To identify the mechanisms that can regulate the development of airway mucosa inflammation during EV respiratory infection, we investigated the production of chemokines by human EV-infected respiratory epithelial cells.

**Methods:** Cultures of adenovirus 12-SV40 transformed human bronchial epithelial cells (BEAS-2B) and lung carcinoma epithelial cells (A549) were infected by reference and wild-type respiratory EV strains. The profile and the levels of intracellular expression of C-C and CXC chemokine mRNAs were determined by real-time RT-PCR assays, whereas the levels of extracellular chemokine secretion were assessed by quantitative ELISA techniques.

**Results:** We observed that the infection of the two airway epithelial cell lines by Coxsackie B5, A21 and Echo 30 reference and wild-type viruses induced dose and time-dependent increases in mRNA and protein secretion for RANTES, MCP-1, IL-8 and IL-10. The protein secretion of these chemokines appeared to be significantly increased at 24 or 36 hours post-infection only in cultures treated by low-doses of gamma-INF comparatively to mock-infected cells, and was correlated to the viral genomic replication activity. No significant increases in chemokine mRNA expression and protein secretion were observed in lower epithelial infected by UV-inactivated EV strains.

**Conclusions:** Therefore, the inflammatory process in EV-induced bronchiolitis appears to be initiated by a replicative infection of lower epithelial cells, and sustained by mechanisms driven by gamma-INF allowing the release of C-C and CXC chemokines.

**P25** Development of a real-time NASBA for respiratory syncytial virus

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**Objective:** The aim was to develop and evaluate a Respiratory Syncytial virus assay based on NASBA amplification and including real-time detection with molecular beacons.

**Methods:** RSV RNA is isolated using a semi automated magnetic extraction method and the NucliSens<sup>®</sup> miniMag. An internal control is added to the sample prior to nucleic acid extraction. Primers are directed against the F-gene region of the RSV genome and both RSV A and B and the internal control are amplified with the same primer set. One generic molecular beacon probe is designed to detect both RSV A and B. An additional beacon is designed for the detection of the internal control. Amplification reactions were performed in a NucliSens<sup>®</sup> EasyQ Analyser allowing real-time detection.

**Results:** Using serial dilutions of in vitro RNA, the 95% hit rate of the real-time RSV assay was found to be 62 copies in isolation for both RSV A and RSV B. Using tissue culture samples, the assay sensitivity was approximately 0.01 TCID50 in isolation for RSV A and 0.1 TCID50 in isolation for RSV B. No cross reactivity was observed with PIV2, PIV3, hMPV A1, hMPV A2, hMPV B1, hMPV B2, or measles. With the real-time assay, RSV in nose swab samples is detectable within approximately 3.0 hours.

**Conclusions:** The data showed that the real-time RSV assay is a rapid and sensitive qualitative assay for the detection of RSV. The use of standardized reagents offers considerable benefits in a routine setting for the clinical management of patients with RSV infections.