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### Oral presentations 3

### OP3-1

### Evaluation of Clart<sup>®</sup> Pneumovir DNA arrays for the detection of respiratory viruses among children hospitalised in intensive care unit

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We investigated respiratory infections among children hospitalized in intensive care unit, using the Clart® Pneumovir DNA array assay (Genomica, R-Biopharm, France). This test allows the simultaneous detection of 11 respiratory viruses (17 types and subtypes): Influenza A, Influenza B, Influenza C, Parainfluenza (1, 2, 3, 4a and 4b), RSV (A and B), Rhinovirus, Adenovirus, Echovirus, Bocavirus, Coronavirus, Metapneumovirus (A and B). This technique was compared to real time PCR detection of influenza A, influenza B and hRSV (ProFlu-1® Real Time Assay from Prodesse, Argène, France) associated with real time PCR detection of rhinovirus (in house PCR). From December 2008 to March 2009, 77 specimens were tested. 60 samples were positive (78%): 45 hRSV (75%), 14 rhinovirus (23%), 8 bocavirus (13%), 6 adenovirus (10%), 2 influenza A, 2 parainfluenza, 2 metapnuemovirus, 1 influenza B and 1 enterovirus. Considering the four viruses detected by both strategies (hRSV, rhinovirus, influenza A and influenza B), 38 viruses were detected using real time PCRs and 67 with Clart® Pneumovir. The overall sensitivity of the 2 real time PCRs (Proflu-1<sup>®</sup> + rhinovirus) was 59% compared to 98% for Clart<sup>®</sup> Pneumovir. In conclusion, Clart<sup>®</sup> Pneumovir DNA array allowed a broader detection of respiratory viruses and revealed a higher sensitivity than real time PCRs including Proflu-1<sup>®</sup> and in house rhinovirus PCR. Nevertheless, real time PCRs are faster and thus easier to implement for routine diagnosis, but Clart® Pneumovir may be useful regarding severe viral respiratory infections.

#### **OP3-2**

# Spread and evolution of avian influenza virus in poultry and wild-birds in Africa

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In Africa HPAI H5N1 virus was first detected in Northern Nigeria and since then in 10 other African countries. Phylogenetic analysis and substitution rates of complete genome sequences of Nigerian strains from the South-West and the North showed that three sublineages were present in Nigeria as early as February 2006 suggesting three independent introductions of H5N1. All three sublineages belong to clade 2.2. and include all African strains with a distinct geographic distribution. H5NI was also identified in hooded vultures found dead or sick throughout Ouagadougou in 2006. The infection of scavenger birds in Africa is likely to cause spill-backs from poultry to wild birds. In 2007 a series of reassortments emerged with a distinct bias of preferred genes, partially replacing the old lineages. In all reassortants, non-structural genes were derived from a distinct sublineage of clade 2.2. viruses. Since the high prevalence of reassortants was typical for West Africa in 2007, the absence of such reassortants anywhere else suggests that reintroductions of influenza A (H5N1) from Africa back to Eurasia must be rare. AIV surveillance in wild birds reveals an African/Eurasian gene pool with clusters that seem to have a higher propensity to develop HPAI strains.

#### **OP3-3**

# Effect of rTGF-β on nitric oxide synthase (iNOS) AND dsRNA dependent protein kinase (PKR) during experimental influenza infection in mice

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**Background:** Influenza virus infection activates the interferon inducible gene, iNOS and PKR. PKR activates cytokine induced apoptosis, however production of nitric oxide inhibits the apoptosis via nitrosylation of caspase-3. This process is regulated by a network of cytokines. Among these, TGF- $\beta$  is known to suppress IFN expression. NO has been shown to contribute to the pathogenesis of influenza virus via modulationg the apoptosis.

**Methods**: Eight-week-old BALB/c mice were intranasally instilled with influenza virus(A/Udorn/317/72/H3N2),  $4.1 \times 10^3$  PFU in 50µl of allantoic fluid or mock infected. rTGF- $\beta$ 1 administered to mice by intravenous injection of 0.5µg/Kg body weight of mouse. IFN- $\gamma$  in BALF, iNOS and PKR expression in the lung homogenate and alveolar lavage cells assayedrespectively. Caspase-3 assay and DNA fragmentation in alveolar lavage cells assayed.

**Results:** Increase in caspase3 activity and DNA fragmentation on 3rd and 5th day however rTGF-  $\beta$ 1 reduced the DNA fragmentation on day 3rd and 5th. Significant increase of INF- $\gamma$  observed on 3rd and 5th day and decreased to basal level on 7th day. Simultaneous administration of rTGF- $\beta$ 1 with virus inhibited release of INF- $\gamma$  on 3rd and 5th day. iNOS expression was detected on 3rd day and maximum level observed on 5th day in virus group. Administration of rTGF- $\beta$ 1 with virus reduced the level of iNOS on 3rd, 5th and 7th day. PKR expression also inhibited by rTGF- $\beta$ 1.

**Conclusions**: rTGF- $\beta$ 1 acts as an immunomodulatory cytokine and inhibits cytokine mediated apoptosis by modulating inflammatory cytokine INF- $\gamma$ , which in turn down regulated the iNOS and PKR. Downregulation of PKR decreased the apoptosis

### **OP3-4**

# Alternative method for airborne virus detection in only few hours / innovative microbial air sampler

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In the context of environmental contamination control and bio-sample preparation, Bertin Technologies (France) has developed a range of laboratory equipments based on new technologies dedicated to collection and sample preparation.

One of these technologies is dedicated to the monitoring of airborne bioparticles. The goal is to propose a sampling method compatible with Rapid Microbiological Methods in order to get rapid, reliable and specific data on airborne biological agents and go beyond impaction method limits.

With this cyclonic technology, airborne particles are separated from the air and collected into a sterile liquid media. This patented solution Coriolis<sup>®</sup> is directly compatible with rapid analysis such as immunoassay, PCR assay, phase cytometry and also standard culture methods...This sampler is validated according ISO14698–1 (Health Protection Agency HPA, Porton Down, UK).

This technology aims at going beyond the traditional impaction method (impaction on agar plates) in terms of time-to-result, more information than only cultivable flora (VNC, viruses, allergens ...) and no saturation of the collection media.

With the Coriolis<sup>®</sup> technology, many studies have been carried out for the sampling of airborne bio-particles to detect bacteria, virus, pollens, allergens or non-cultivable pathogens with rapid microbiological methods as PCR analysis (*Pneumocystis*, Respiratory Syncytial Virus (RSV), bacteriophage, *Legionella, Stachybortrys chartarum* ...). Specific results on viruses' applications can be presented.

### OP3-5

## Possible independent replication of HBV in the CNS of HIV-1 infected patients

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**Background and Objective**: Coinfection with HBV has a high frequency in Romanian HIV infected patients. As experience regarding the neurotropic character of hepatitis B virus (HBV) is limited to several case reports, the aim