Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

for HAdV DNAemia (HR 9.7; 95% CI 3.4–27.4; p = 0.000). HadV DNAemia was a predictor for allo-reactive disease (HR 2.6; 95% CI 1.2–5.4; p = 0.013).
Conclusions: HAdV positivity in NPA pre-HSCT is a very strong predictor for the development of HAdV DNAemia after HSCT. Early detection and intervention might contribute to prevent HAdV-disease after HSCT.

Oral presentations 4

OP4-1
Surveillance and oseltamivir resistance of human influenza A virus in Turkey during the 2007–2008 season

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Monitoring the activity of influenza viruses is important for establishing the circulating types and for detection of the emergence of novel sub-types and antiviral resistant strains. This is the first report from Turkey on the surveillance and oseltamivir resistance of influenza viruses in 2007–2008. Five hundred and twenty four nasal swabs were tested from different geographical regions in Turkey during November 2007 – April 2008. One hundred and sixty three (31%) samples were positive for influenza viruses of which 111 (68%) were influenza A, 52 (31%) influenza B using an immuno-capture ELISA. Forty isolates were selected at random from influenza A positive samples and grown in MDCK cell cultures. The supernatant of the cell cultures was used for RNA extraction followed by RT-PCR to detect the sub-types. Sub-typing revealed all samples as A/H1N1. The N1 gene segment of 30 A/H1N1 samples was sequenced in part, from the 201st to 365th residue, which included the critical region for oseltamivir resistance. Then resulting sequences were analyzed with oseltamivir sensitive and resistant strains obtained from National Center for Biotechnology Information (NCBI) GeneBank by CLC Main Workbench Software. H275Y (H274Y according to N2 numbering) mutation, which is known to confer resistance to oseltamivir, was detected in 6 out of 30 (20%) H1N1 isolates. The D354G mutation was observed in all oseltamivir resistant H1N1 isolates but not in the oseltamivir sensitive isolates. Assay of neurominidase activity revealed that these isolates were resistant to oseltamivir, but sensitive to zanamivir.

OP4-2
Timely diagnosis of respiratory tract infections: evaluation of the performance of the respifinder assay compared to the RVP assay

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Lower respiratory tract infections are a common cause of hospitalization in infants and young children and are typically caused by viral or bacterial pathogens. Diagnostic methods such as culture and serology are time consuming and have several other drawbacks such as limited sensitivity, long turn-a-round time and limited number of detected pathogens. Nucleic acid amplification methods can increase sensitivity and enable the initiation of appropriate interventions without delay.
Broad-spectrum detection and identification circumvent the use of individual diagnostic DNA or RNA based assays. At present, several commercial assays are available for broad-spectrum detection. We compared the performance of the Respiratory Virus Panel assay (RVP) (Luminex Molecular Diagnostics) with that of the Respifinder assay (Pathofinder) for the detection of respiratory pathogens. A total of 106 EQC samples of 9 QCMD Quality Control panels were analysed, of which 95 samples were expected to be positive.
RVP was positive in only 31 samples. For 8 samples an inconclusive result was obtained. All samples containing adenovirus, Coronavirus NL63, Coronavirus OC43 and Coronavirus 229E were false negative with RVP. No false positive results were found. Hence, sensitivity was 32.6% and specificity 100%.
A positive result was found with the Respifinder assay in 75 samples. For 3 weak positive samples an inconclusive result was obtained. For 2 adenovirus type 31 samples the analysis was also false negative. No false positive results were found. Hence, sensitivity was 79% and specificity 100%.
In conclusion, the results of our study seem to indicate a better sensitivity for the Respifinder.

OP4-3
Molecular detection of respiratory viruses: routine application on 522 samples taken in children less than 2 year old

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We describe the development of two multiplex RT-PCR methods for the detection of respiratory viruses in a routine laboratory which tests about 3000 respiratory samples per year. We specifically targeted children less than two years of age. The detection of hRSV, Influenza A, Influenza B was performed every day by multiplex real time RT-PCR (ProFlu-1 Real Time Assay from Prodesse, Argène, Verniolle, France) and the detection of Rhinovirus was performed twice a week by real time RT-PCR (Andeweg et al., J Clin Microb 1999). Between January and April 2008, 522 respiratory specimens were tested, 309 were positive (59.2%). Single infections were detected in 272 samples: 126 rhinovirus, 109 hRSV, 20 influenza B and 17 influenza A. Dual or triple infections were detected in 37 samples, mainly with hRSV and rhinovirus (29 cases). Ct values were comparable for all 4 viruses between single and multiple infections. Clinical data were collected for 133 patients. Concerning children below 42 days of age (38 patients), 17 presented with bronchiolitis and 14 were positive (82%). Regarding children over 42 days of age (95 patients), 38 presented with bronchiolitis and 29 were positive (76%). Implementation of molecular detection of hRSV, influenza A, influenza B and rhinovirus largely improved virus detection compared to classical technics: +15% for hRSV and +93% for rhinovirus. Nethertheless, about 40% of the samples remain negative which emphasizes the need of a broader detection of other respiratory viruses.

OP4-4
Prevalence, quantification and cut-off level of parvovirus B19 DNA in endomyocardial biopsies of cardiac patients and non-cardiac control subjects

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Purpose: Parvovirus B19 (PVB19) persistence has been linked to acute myocarditis and dilated cardiomyopathy. Unfortunately, previous studies lack detailed research on virus presence in normal hearts and various forms of heart failure. Therefore, we evaluated in detail viral DNA prevalence in hearts of cardiac patients and controls.
Methods: Endomyocardial biopsies (EMBs) were collected from patients with diseased hearts and compared to controls. Real-time PCR reactions for PVB19, adenovirus, human herpesvirus-6 and Epstein-Barr virus were performed using ABI prism 7000 and standard curves were included in each run.
Results: A high prevalence of viral genome could be amplified in both groups, up to 84%. Importantly, a significant higher PVB19 DNA load was detected in patients with diseased hearts compared to control subjects, whereas other viruses did not reveal any significant differences in DNA loads. In cardiac patients, mean PVB19 DNA load was 450 copies/ug DNA with a lower 3SD range of 273 copies/ug DNA. In control subjects, mean PVB19 DNA load was 144 copies/ug DNA with an upper 3SD range of 231 copies/ug DNA. Therefore a cut-off value of 250 copies/ug DNA was most optimal to indicate increased PVB19 loads associated with cardiac disease.
Conclusions: The present study demonstrates high virus prevalence in various forms of heart failure and healthy hearts. Importantly, high PVB19 loads in EMBs are associated with cardiac disease. We propose the use of a cut-off value of 250 copies/ug DNA to discriminate between normal PVB19 DNA levels and increased PVB19 DNA levels associated with cardiac disease.

OP4-5
Genetic variants of human parvovirus B19 in South Africa: co-circulation of three genotypes and identification of a novel subtype of genotype 1.

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Background: Three distinct genotypes of parvovirus B19 are now recognized (1, 2 & 3), which differ in overall DNA sequence by ~10%. The

distribution of B19 genotypes in South Africa has not before been examined.

Methods: Two hundred and fifty samples submitted to a diagnostic virology laboratory for parvovirus DNA detection were analyzed retrospectively. Screening for parvovirus DNA in these samples had been performed using primers specific to B19 genotype 1. Sufficient sample was available from 141 PCR-negative and 16 PCR-positive serum and bone marrow specimens for further analysis. These samples were tested by means of a consensus NS1 nested PCR able to amplify all three parvovirus B19 genotypes. PCR products were sequenced and analyzed phylogenetically by comparison with reference sequences.

Results: Of the 64 PCR positive samples investigated, 50 (78.1%) were identified as genotype 1 by genotype-specific PCR or consensus NS1 PCR and sequencing; 3 (4.7%) as genotype 2 and 11 (17.2%) genotype 3 by analysis of NS1 sequences. Furthermore, phylogenetic analysis identified two genotype 1 sequences which were distinct from the previously described genotypes 1 A and 1B.

Conclusion: In this cohort, all three genotypes were detected, with genotype 1 predominating as anticipated. Furthermore, we provide evidence for the recent circulation of genotype 2 viruses, and the presence of novel genotype 1 viruses in South Africa. The diversity of parvovirus B19 viruses has important implications for, amongst others, the laboratory diagnosis and treatment of these infections.

OP4-6
The polyomavirus BK agnoprotein co-localizes with lipid droplets

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The human polyomavirus BK (BKV) infects up to 90% of the general population. In immunosuppressed individuals, it is the major cause of polyomavirus-associated nephropathy in kidney transplant and hemorrhagic cystitis in hematopoetic stem cell transplant patients. Despite the known clinical impact of BKV replication in those patients, effective antiviral therapy is lacking. Agnoprotein, a non-structural viral protein of only 66 amino acids is abundantly expressed in the cytoplasm during the late phase of the BKV life cycle but its precise function is unknown.

Immunofluorescence microscopy revealed two prominent distributions in the cytoplasm, a fine granular one with perinuclear enrichment and an accumulation around donut-like structures. Using BKV(Dunlop)-infected or agnoprotein-transfected cells, we systematically performed co-localization studies with markers of cellular structures as a first step to elucidate the function of agnoprotein (panel A). We found that agnoprotein co-localizes with lipid droplets in a dynamic fashion in human renal proximal tubular epithelial cells, where BKV replicates in vivo as well as in other cells that support BKV replication in vitro (UTA, Vero cells) (panel B, green LipidTox stain of lipid droplets, red agnoprotein, yellow merge). We also observed that lipid loading of cells interferes with BKV replication. Analysis of several truncated agnoprotein mutants fused to GFP suggests that amino acids 20–42 are involved in the targeting to lipid droplets.

The interaction of polyomavirus with lipid droplets is novel and its implication for the interplay between virus and host cell will be discussed.

OP4-7
Polyomavirus JC (JCV) with rearranged noncoding control region are found in cerebrospinal fluid, but not in urine and increase viral early gene expression

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JCV infects ~60% of the general population persisting in the renourinary tract. The viral genome contains the noncoding control-region (NCCR) with origin of replication and promoter/enhancer functions driving early and late gene expression. In immunocompromised patients, JCV can cause progressive multifocal leukoencephalopathy (PML), a rare, usually fatal disease of the CNS. In PML tissue, rearranged (rr)-NCCR have been detected but their impact is unclear. JCV-NCCRs were amplified from urine, plasma, and cerebrospinal fluid (CSF) of JCV-positive HIV/AIDS-patients, sequenced, and cloned into a dual-reporter vector. After transfection, transcription of early (RFP) and late (GFP) region was evaluated by fluorescence microscopy. Recombinant JCV were generated by replacing the original NCCR with the amplified NCCRs in the context of the JCV(Mad-4) genome. Infectivity and viral load was determined in PDA cells. NCCR isolated from urine demonstrated archetype (ww)-architecture. However, we found rr-NCCRs in plasma and CSF. Our reporter assay revealed that ww-NCCRs were associated with low early/late gene expression. In contrast, rr-NCCRs were characterized by increased early gene expression. Recombinant JCV with rr-NCCR replicate more efficiently in PDA cells than ww-NCCR JCV. JCV-specific antibodies against JCV virus-like particles were high, but JCV-specific cellular immune responses were not detectable suggesting a failing cellular immunity is a prerequisite for emergence of more pathogenic rr-NCCR variants.

In conclusion, naturally occurring rr-NCCRs show increased early gene expression compared to ww-NCCR. JCV-specific cellular immunity is a likely key barrier to JCV variants with increased early gene expression in brain tissues, which is failing in PML.

OP4-8
Virus discovery 454 sequencing

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Virus discovery cDNA-AFLP (VIDISCA) is a virus identification method that can amplify both RNA and DNA virus without prior knowledge of the sequence. However, cellular compounds such as chromosomal DNA and ribosomal RNA interfere with the reaction by acting as competitors. Due to this VIDISCA can only be used with high viral load and/or low background DNA/RNA in these samples. 454-Sequencing is a new technique that can sequence 400.000 fragments with a length of 200 to 300 nucleotides in a 6 hour run. Furthermore, it can be expanded with 12 Multiplex Identifiers Adaptors (MID) which are 12 unique keys of 10 nucleotides long recognized by the software. With these MIDs 12 samples can be pooled into a single sample allowing more samples to be amplified in a single run. We have adapted the VIDISCA method such it can be used for 454-sequencing with MID (VIDISCA-454). We tested this technique using 12 respiratory samples for viral identification. For 6 out of 12 samples a viral pathogen was identified. Viral sequences per sample were present between 1.1 and 0.06%. Besides the identification of these viruses in respiratory samples, preliminary results show that viruses can be identified in both CSF as well as blood-plasma. This is due the background DNA/RNA which is more abundant present in respiratory samples than in CSF or blood-plasma.

OP4-9
DECIBEL-study: congenital cytomegalovirus infection in young children with bilateral permanent hearing

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Background: A significant number of asymptomatic newborns infected with congenital Cytomegalovirus (CMV) will present with permanent childhood hearing impairment (PCHI) during early childhood.

Objectives: The role of congenital CMV infection in causing PCHI in the Netherlands was investigated. Furthermore, the efficacy of two different hearing screening strategies and the developmental outcome were assessed.

Study design: We included 192 children with PCHI, who were offered a hearing screening in their first year of life and were known at an Audiological Center with PCHI at the age of 3–5 years. Dried blood spots from 171 children