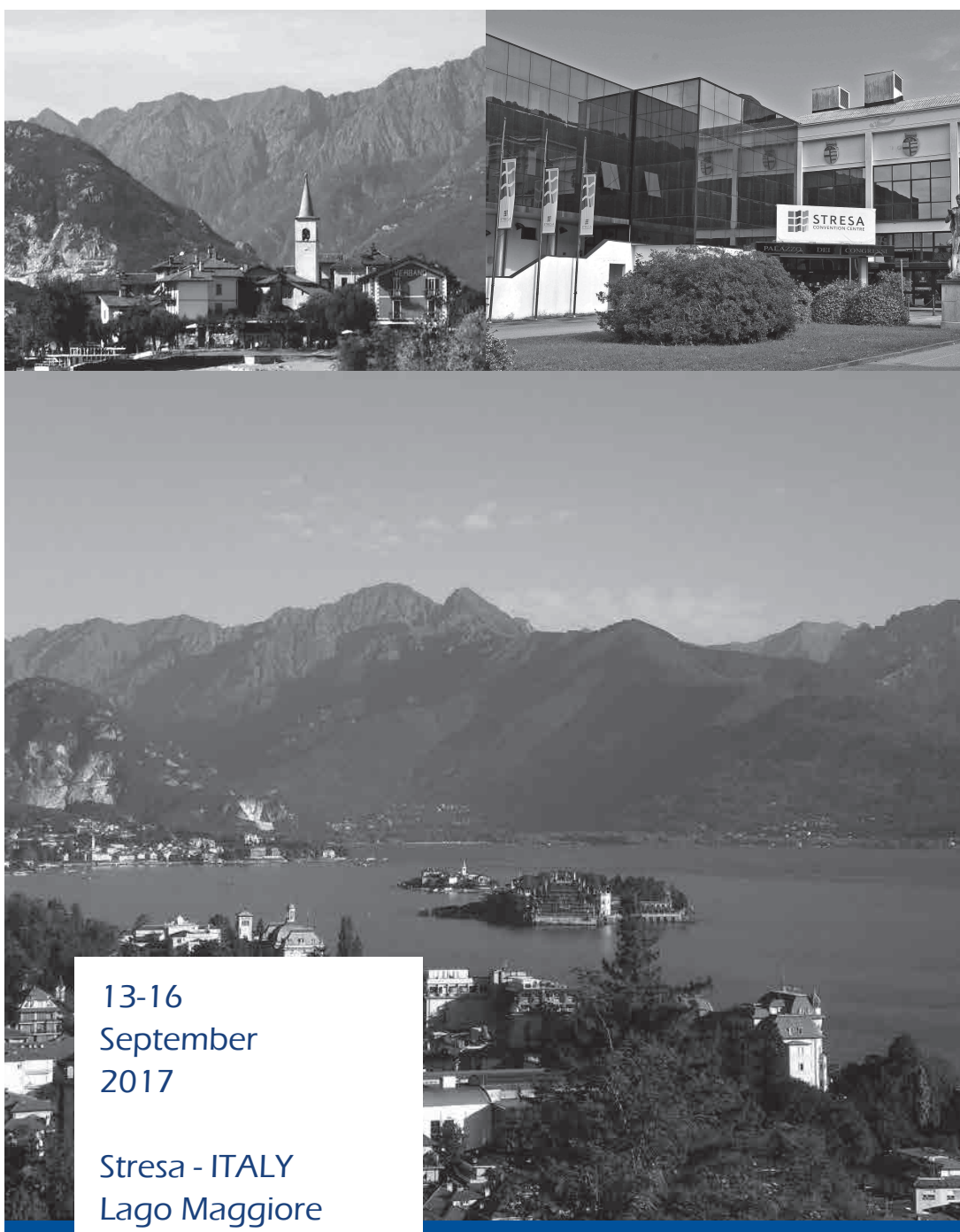




# 20th ESCV 2017

## ANNUAL MEETING

A B S T R A C T   B O O K



13-16  
September  
2017

Stresa - ITALY  
Lago Maggiore

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# KEYNOTE LECTURES

Wednesday, September 13, 2017

Session: Respiratory viruses

## ENTEROVIRUS D68 EPIDEMIC IN EUROPE; A STATUS UPDATE

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Circulation of enterovirus D68 (EV-D68) in 2014 and 2016 temporally and geographically coincided with increases in cases of acute flaccid myelitis (AFM). The identification of EV-D68 in respiratory specimens of these AFM cases suggest an association, but the definitive proof has still to be made, although there is accumulating epidemiological and biological evidence for this causal interplay between EV-D68 and AFM. Collaboration between virologist and clinicians has characterized the patients and provide information on long-term outcome as well shown that crucial information is lacking in some countries. Furthermore, a collaboration between European and US clinicians and virologists has been initiated to provide more epidemiological, diagnostic and clinical data and to learn from each other, for the benefit of our primary stakeholder, the patient. The latest information will be presented hoping that we are ready for the next EV-D68 upsurge.

Session: Emerging viruses

## ONGOING AND EMERGING ARBOVIRUS THREATS

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Arthropod-borne viral infections are caused by viruses that are transmitted among humans by the bite of an infected arthropod, mainly mosquitoes and ticks. The past decades have seen an unprecedented emergence of epidemic arboviral diseases, characterized by global spread and high morbidity. Recent examples include the yellow fever outbreak that occurred in Angola, with hundreds of deaths, the epidemics of Zika virus infection in the Pacific area and the Americas and the introduction of chikungunya virus into the Caribbean and Latin America, where both viruses caused over one million cases of infection. In addition, the worldwide incidence of dengue has exponentially increased in the recent years, associated with geographical expansion of endemic areas and co-circulation of the four serotypes, increasing the risk of severe disease. In Europe, tick-borne encephalitis, West Nile, and Crimean-Congo haemorrhagic fever viruses have changed their geographical distribution and human infections continue to increase. Drivers of arbovirus emergence include urbanization, globalization, international travel, and climate change. These factors have facilitated the spread of vectors, the introduction of arboviruses into new areas, and transmission in urban environments. On the other hand, arboviruses have rapidly adapted to new ecological

niches, increasing their transmissibility and virulence. Clinical presentations of arboviral diseases, characterized by febrile syndromes and less frequently haemorrhagic or neurological complications, are generally undistinguishable. Laboratory testing is crucial for etiological diagnosis, which relies on molecular methods, because of the extensive cross-reactivity of serological assays. Rapid point-of-care tests and syndromic approaches are also needed because of the frequent co-circulation of different arboviruses. Strategies to combat arboviruses are based on the combination of vector control programmes, use of personal protective measures, and vaccination of at-risk populations. However, notwithstanding advances in basic, translational, and clinical research, many questions remain to be answered, making arboviral epidemics and related disease still unpredictable and unpreventable events.

Thursday, September 14, 2017

Session: Viruses in immunocompromised patients

## OPPORTUNISTIC VIRAL INFECTIONS BEYOND HERPESVIRUSES

H.H. Hirsch

Session: Hepatitis viruses

## HCV IN THE DAA ERA

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The excellent efficacy of the therapy of HCV infection with Directly Active Antivirals (DAA) led to overlooking the capacity of HCV to escape antiviral pressure, and generate resistance to DAA. Today, with the new generation of DAA available in clinical practice, around 95% of treated patients reach SVR12 (sustained virological response, i.e. negativity of HCV viral load at 12 weeks-time after the end of treatment), that, driven by the genetic structure, the cellular distribution, and the dynamics of the replication of HCV, corresponds in nearly all cases to the cure of this infection.

HCV is a flavivirus provided with an RNA polymerase lacking proofreading function.

The tumultuous replication, together with the mistakes done during replication by RNA polymerase, make very common the generation and selection of mutations within viral genome. If this phenomenon occurs during incomplete/insufficient pressure of DAAs, the chances to generate and select viral quasiespecies resistant to DAA are very high. Indeed, in clinical practice, while the number of failing patients is limited (about 3-4% in percentage; however, this number reaches about 3-4,000 patients if related to the number of those treated with DAA), those carrying resistance to DAA exceeds 60-70% of the total (far more than HIV treatment under similar conditions). The mutations frequently

cause a broad resistance to the drug class (making the entire class difficult to be used in second line), and, particularly those in NS5A, tend to remain for long time in viral genome, due to their high fitness, therefore hampering the future use of this class at the time of second regimen.

Taken all together, clinical and virological data strongly suggest that the therapy of HCV infection split in two parts the patients treated with DAAs.

On one side, those that are cured by therapies properly prescribed (time, drug association, use of ribavirin, etc); on the other side, those that fail therapy with resistance that might heavily affect chances of successful retreatment.

For all these reasons, the best option for increasing the chance of virological success of first line therapies toward nearly 100% (a reachable target) is to treat at best with first line regimens, by using diagnostic tools whenever possible, in order to choose at best the drug regimens for each single patient. Tailoring therapy remains the most appropriate approach, also in HCV-infected patients.

**Session: HIV and other retroviruses**

## **LONG TERM CONTROL OF HIV INFECTION: PERSPECTIVES AND CHALLENGES**

G.M. Gatell

Friday, September 15, 2017

**Session: Virus-associated tumors**

## **HPV MALIGNANCIES BEYOND CERVICAL CANCER. AN UNIVERSAL VACCINATION APPROACH REQUIRED?**

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Approximately 40 human papillomavirus (HPV) types are known to infect the mucosal epithelium, with a subset of 10 to 15 high-risk HPV types being associated with lesions that can progress to cancer. These HPV types are the etiological agents of virtually all cervical carcinomas and their immediate precursors — high-grade cervical intraepithelial neoplasia (CIN) lesions. In addition to cervical carcinoma, high-risk HPV types, the most frequent being HPV-16, play the leading etiological role in the development of anal cancer and a substantial proportion of vaginal, penile, vulvar and oropharyngeal (mainly tonsillar) cancers.

Currently, there are three licensed prophylactic HPV vaccines: 2-valent, 4-valent, and 9-valent HPV vaccine. All three HPV vaccines are based on synthetically manufactured virus like particles (VLPs) using recombinant expression of the L1 epitope in yeasts and insect cells. Following extensive evaluations in clinical trials, the first decade of routine HPV vaccine use provides overwhelming evidence of the vaccines' safety and their real-life effectiveness. The impact of HPV vaccination in real-world settings has become increasingly evident, especially among girls vaccinated before HPV exposure in countries with high vaccine uptake. Maximal reductions of approximately 90% for HPV infection with vaccine-targeted types, approximately 90% for genital warts, approximately 45% for low-grade cytological cervical abnormalities, and approximately 85% for high-grade histologically proven cervical abnormalities have been reported. These declines were evident not only in vaccinated females, but also in unvaccinated females and males, strongly suggesting herd protection. In addition to cervical lesions, high efficacy in preventing high-grade precursors of vulvar, vaginal and anal cancer related to vaccine HPV types (and genital warts in the

case of 4-valent and 9-valent vaccines) has recently led to a paradigm shift of HPV vaccine from a female-only cervical cancer vaccine to a vaccine for the prevention of several HPV-related diseases and cancers in both genders. HPV vaccination has the great potential to dramatically reduce incidence and mortality of all HPV-related neoplasms; in fact, no other neoplastic diseases can currently rival the magnitude of this potential.

**Session: Immune response to viral infections**

## **TYPE I INTERFERON IN VIRAL INFECTIONS: A RECONSIDERATION OF ITS ROLE 60 YEARS AFTER THE DISCOVERY**

G. Antonelli<sup>1</sup>

<sup>1</sup>Department of Molecular Medicine and University Hospital Policlinico "Umberto I" Sapienza University, Rome Italy

Interferon (IFN) system consists of a broad family of cytokines that have a pivotal role in the development of both innate and adaptive immune response. According to their cellular origin and the type of receptors they bind to, IFNs are grouped into three different types. Type I IFN is comprised of multiple species, all of which signal through the same heterodimeric receptor. In humans, there are at least twelve subtypes of IFN $\alpha$ , one IFN $\beta$ , one IFN $\omega$ , one IFN $\epsilon$ , and one IFN $\kappa$ . The action of type I IFNs promotes the expression of a multitude of IFN regulatory factors (IRFs) and IFN-stimulated genes (ISGs) which in turn leads to the recovery from viral infections through several direct (antiviral proteins) and indirect (immunomodulation) activities. Although the role of type I IFN in the protection against viral infections is consolidated and has been studied for decades, these cytokines are now emerging as key drivers of sustained inflammation and immune activation. Then, it is clear, now more than before, that prolonged type I IFN signalling can lead to immune dysfunction potentially promoting the persistence of viral infection. In conclusion, the role of type I IFN in recovery from viral infections is still not well defined, and is potentially much more complicated than expected.

**Session: Virus-associated neurologic syndromes**

## **VIRAL INFECTIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM**

H.J. Harvala

Viruses have become the major cause of meningitis in Europe where mumps and bacterial causes have been effectively controlled by vaccination. Viral meningitis occurs most commonly in young children and adults under the age of 40 years, and is usually caused by enteroviruses. In addition, the related parechoviruses are increasingly recognised as another significant cause of meningitis in very young children. Acute viral encephalitis is rarer, but often more devastating disease entity than meningitis. Herpes simplex virus is the most common cause of sporadic encephalitis; it is usually seen in people older than 65 years of age and without treatment results in death in 70% of cases. The other causes of encephalitis include enteroviruses, VZV and various arboviruses (i.e. TBE in Europe).

A total of 116 enterovirus types have been identified from humans, and of these, 45 have been discovered in the last ten years. From these, species B enteroviruses including CBV1-6, CAV9 and echoviruses (notably types 6, 9, 11 and 30) and species A enteroviruses including EV-A71 have been associated with meningitis and encephalitis in Europe.

In addition to the central nervous system infections, viruses play a significant but often under-recognized role in the development of the peripheral nervous system infections. These include polyneuropathies associated with HIV and

HTLV infections, radiculitis and neuralgia following HSV and VZV infections as well as acute flaccid myelitis with species D enterovirus, EV-D68. Furthermore, knowledge about the spectrum and magnitude of neurological disease associated with hepatitis E virus is rapidly increasing.

Epidemiology, pathology, diagnosis and potential treatment of common and recently emerging viral infections of the central and peripheral nervous system will be discussed.

#### Session: Gastrointestinal viruses

### HUMAN MIGRATIONS, SUSCEPTIBILITY AND GASTROINTESTINAL VIRUSES

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<sup>1</sup>Department of Microbiology, Faculty of Medicine, Hospital Clínico Universitario, University of Valencia, Valencia, Spain

Human migrations occur continuously, allowing the spread of infectious agents globally. Despite the efficacy of the group A rotavirus (RVA) vaccines, many children still suffer RVA infections. Occasional interspecies transmission and genomic reassortments between human and animal RVAs contribute to their genetic diversity. During the last decades, different RVA genotypes like G9P[8] and G12P[8] emerged and spread worldwide. The high similarity between the sequence of the major outer capsid VP7 gene of human and porcine G12 isolates suggested that human strains may have arisen through reassortment with porcine strains. More recently, emergence and spread of G3P[8] rotaviruses with an atypical equine-like VP7 and a DS-1-like genetic background have been reported. Phylogenetic studies of these G3P[8] strains demonstrated that they are reassortants of G1P[8] human and equine-human G3P[8] strains with a DS-1-like genetic backbone identified in 2013 in Asia and Australia. These novel equine-like G3 RVAs have rapidly spread around the globe. Noroviruses (NoVs) are the main cause of foodborne diseases and they show a very high genetic diversity. Most NoV infections are caused by different variants of the GII.4 genotype. In the last two decades, GII.4 genotype has caused six pandemics of acute gastroenteritis. These pandemics spread rapidly across the globe, causing great economic burden due to medical and social expenses. During the winter season 2014-15, a novel NoV GII.P17\_GII.17 variant, Kawasaki 2014, emerged in several countries in Asia, replacing the previously dominant variant, GII.4 Sydney 2012. RVAs and NoVs recognize human histo-blood group antigens (HBGAs) as ligands for attachment to the enterocytes. HBGAs containing ABO, secretor (H type 1) and Lewis antigens are highly polymorphic. The host genetic profile can determine the evolution and spread of new strains. It has been reported that secretor individuals with a functional FUT2 gene are more susceptible to P[8] RVAs than non-secretors. Host genetics may be important for the susceptibility to RVA and NoV infections. Further investigations are needed to clarify how host genetics influences susceptibility to these infections and whether genetic background is a factor determining RVA vaccine uptake.

## Saturday, September 16, 2017

### Session: Viral infections in pregnancy

#### ZIKA VIRUS INFECTION IN PREGNANT WOMEN

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#### BACKGROUND

Zika virus (ZIKV) infection has been linked to complications in pregnancy, and severe ocular and neurological deformities in neonates born to ZIKV-infected mothers including microcephaly have been reported in Central and South America and the Caribbean. Given the overlapping presence of Dengue virus (DENV) in the majority of ZIKV epidemic regions, implications for protection and pathology warrant careful investigation.

#### PATIENTS AND METHODS

From February 2015 to April 2017, 19 active ZIKV infections (14 non-pregnant and 5 pregnant) were investigated in Pavia, Italy. The diagnostic work-out included: a) the determination of ZIKV IgM and IgG; b) DENV 1-4 IgM and IgG in serum samples along with; c) confirmation of serological results by neutralization assay for ZIKV and DENV 1-4, and NS1 Blockade-Of-Binding (BOB) ELISA Assay; d) the determination of DENV RNA and ZIKV RNA in plasma, saliva, urine, amniotic fluid and fetal blood using both a pan-Flavivirus heminested RT-PCR as well as virus-specific Real-time RT-PCRs, and e) sequencing of positive pan-Flavivirus amplicons; f) Elispot assay for DENV 1-4 and ZIKV

#### RESULTS

Among the five pregnant women, three were DENV-ZIKV immunologically cross-reactive, and two were DENV-naïve. ZIKV RNA was detected after onset of maternal symptoms up to: 198 days in plasma, 154 days in urine, and 34 days in saliva. The three cross-reactive mothers did not transmit ZIKV to the fetus, while the two non cross-reactive mothers transmitted ZIKV to the fetus. In one case, the presence of ZIKV in brain tissues of an aborted asymptomatic fetus, as well as in the amniotic fluid of pregnant woman was documented. In the second case, a microcephalic neonate was delivered.

#### CONCLUSIONS

The potential impact of DENV-ZIKV cross-reactivity on the protective efficacy of ZIKV-induced antibody response needs to be carefully investigated

### STANDARDIZATION OF RUBELLA VIRUS IMMUNOASSAYS

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Immunity to rubella is usually determined by measuring the rubella specific IgG (RV-IgG) with commercial enzyme immunoassays (CIAs) that provide results in IU/mL. The use of IU implies that health professionals assume that results obtained by different CIAs are comparable. Indeed, all CIAs are calibrated with the identical World Health Organization International Standard (RUB-1-94) established in 1996 by the WHO Expert Committee for Biological Standardization. The international collaborative study performed prior to establishment showed the suitability of RUB-1-94 for the harmonization of RV-IgG assays used at that time.

Since, several factors such as highly effective vaccination programs (resulting in an overall reduction in the levels of RV- IgG in vaccinated individuals compared with those acquiring immunity through natural infection), and the development of new technology, have complicated the approach to standardizing RV-IgG CIAs. This situation has clinical consequences as laboratories can report discrepant results to clinicians leading to misinterpretation of results, confusing clinical management, anxiety for the pregnant woman, unnecessary (re)-vaccination, and/or medico-legal complications. This situation has been highlighted by a study published in 2016 investigating rubella immunity of 322 susceptible pregnant women, with eight CIAs, an immunoblot (IB) and a neutralization assay. Results indicated that women were immune when discrepant results between CIAs were observed, and that half women considered seronegative with at least one CIA had in fact protective anti-E1 antibodies (positive IB). However, do these very low levels of RV-IgG protect against rubella? This question has been assessed in a clinical trial investigating both humoral and cell-mediated immunity of pregnant women with negative or equivocal RV-IgG titers. The main result of this study was that women with negative RV-IgG (tested by CIAs) but positive IB, raised a secondary immune response to post-partum vaccination and could therefore have been considered as protected. There has been significant progress in rubella diagnosis but further efforts are required for more accurate determination of the immune status of childbearing age women.

Session: Advancements in diagnosis, monitoring and treatment

### MICRORNAS, NEW MARKERS IN CLINICAL VIROLOGY?

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The discovery of small regulatory non-coding RNAs has been an exciting advance in the field of genomics. MicroRNAs (miRNAs) are short sequences of non-coding RNA which regulate gene expression at the post transcriptional level either through translation repression or mRNA degradation. Viruses utilize this fine tune expression mechanism, either by encoding their own miRNAs or by affecting the host's miRNA expression profile for their own benefit. The altered expression of miRNA level in an infected human can be identified by the use of advanced diagnostic tools. The use of miRNA as an emerging tool for the identification of the human infectious disease is discussed. Several miRNAs have been reported as a molecular biomarkers in infectious diseases caused by viruses of clinical importance, namely, herpesviruses, polyomaviruses, hepatitis B virus, hepatitis C virus, human papillomavirus, and human immunodeficiency virus. The discovery of circulating miRNA in the blood of infected patients has the potential to become a powerful, non-invasive biomarker in coming future and may have a key role in early diagnosis of infection.

### DETERMINING T CELL RESPONSES TO FLAVIVIRUSES

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Flaviviruses are among the most important human pathogens transmitted by arthropods and include Yellow fever (YF), dengue, Zika, West Nile, Japanese encephalitis and tick-borne encephalitis (TBE) viruses, which pose an increasing threat to public health worldwide. Humans infected with flaviviruses develop robust T cell responses and long-term protective neutralizing antibodies directed against the viral envelope protein E. The generation of such antibodies requires the help of CD4 T cells that recognize peptides derived from the viral structural proteins. Elucidating specific protein sites that induce such helper T cell responses provides new insights into the mechanisms of immune protection and will inform the development of immunoassays for evaluating T cell responses elicited by new vaccines. We have developed highly sensitive ELISPOT assays with large peptide libraries for identifying the antigenic sites in the virion proteins targeted by CD4 T cell responses in the context of human infections with Zika, TBE and the live YF vaccine virus. The proteins of flaviviruses are structurally homologous but highly diverse at the amino acid level. Our data reveal that the locations of T cell epitopes within three-dimensional protein structures are strikingly similar between different flaviviruses, consistent with a strong influence of protein structure that shapes CD4 T cell responses. Of the identified T cell epitopes, most are virus type-specific and show no cross-reactivity with other flaviviruses, but others are conserved among flaviviruses and may induce broadly cross-reactive T cells that participate in subsequent responses to heterologous flaviviruses. The use of immunoassays that distinguish between cross-reactive and virus type-specific T cell responses will improve understanding of T cell cross-reactivity in sequential flavivirus infections and its potential impact on protective immunity.

# ORAL PRESENTATIONS

O1

## THE YOUNGEST INFANTS AT THE HIGHEST RISK FOR SEVERE RSV DISEASE: EPIDEMIOLOGY AND MOLECULAR PROFILE OF RESPIRATORY SYNCYTIAL VIRUS (RSV) AMONG YOUNG CHILDREN < 5 YEARS-OF-AGE IN DENMARK, 2010 TO 2015

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### BACKGROUND-AIM

Infections with respiratory syncytial virus (RSV) are highly prevalent, particularly in young infants where RSV is responsible for substantial morbidity and mortality worldwide. Nevertheless, data on RSV disease burden are sparse. We calculated the age-specific incidences using national registry data from Denmark to determine the age-specific incidence and direct medical costs of annual RSV-associated admissions in children < 5 years for the period of 2010-2015. Furthermore, we conducted a separate retrospective laboratory investigation of RSV-RNA PCR positive specimens, to estimate the distribution of RSV subtype A and B in Denmark during the study period 2010-2015.

### METHODS

Calculation of national hospitalization-, microbiology-, and vital statistics data using linkage of registries by unique personal identifier. Direct and indirect estimation of RSV-associated mortality using a 30-day window from hospital discharge to death. For laboratory analyses, we used real time reverse-transcriptase PCR to characterize ~150 randomly selected RSV-RNA positive specimens into subtype A and B, respectively.

### RESULTS

In a population of approximately 325,000 children < 5 years, we identified ~2500 RSV-associated hospitalizations annually amounting to total direct medical-costs of ~EURO 4.3 million per year. The incidence of RSV-associated hospitalizations peaked in infants 1-2 months of age followed by infants 2-3 months of age, and infants <1 months-of-age, respectively. Five RSV-associated deaths were identified. The overall distribution of RSV subtypes among a subset of 158 samples from unique individuals < 5 years of age was 73% subtype A (n=116), 19% subtype B (n=29) and 8% untypeable (n=13). Subtype A dominated in most seasons, namely 2010/11, 2012/13, and 2014/2015.

### CONCLUSIONS

Our findings demonstrate that in a Western country as Denmark, RSV constitute a considerable burden on childhood health. The peak in hospitalizations occurs in the 1-2 month old infants indicating that maternal vaccination likely is the best approach to reduce the high incidence of RSV-associated hospitalizations in young Danish infants. Both RSV subtypes A and B co-circulate with an alternating inter-seasonal pattern among Danish children, and a predominance of subtype A.

O2

## EXPLORING METAGENOMIC NEXT-GENERATION SEQUENCING IN ROUTINE DIAGNOSTICS OF VIRAL RESPIRATORY INFECTIONS

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### BACKGROUND-AIM

Viruses are the main causative agents of respiratory tract infections. However, with current diagnostics, PCR multiplex assays, no causative agent can be found in a large portion of the respiratory tract infections. PCR multiplex assays are restricted by the limited number of predefined targets. Metagenomic next-generation sequencing (NGS) enables unbiased detection of all pathogens in a clinical sample, including possible unknown viruses. However, the optimal pre-sequencing treatment of respiratory samples for optimal sensitivity remains unclear. In this study, we explore NGS as a method for routine diagnostics of viral respiratory infections.

### METHODS

Respiratory samples positive for RNA and DNA viruses by means of multiplex real-time PCR were used to optimise various pre-sequencing workflows, including viral enrichment, internal controls, reproducibility, sensitivity, and specificity. Metagenomic sequencing was performed on a Illumina HiSeq. Subsequently, reads were analysed using the bioinformatics classifier Centrifuge. After determining the optimal strategy for the detection of viral pathogens, 25 variable respiratory samples of paediatric patients and 88 nasopharyngeal swabs of COPD patients were tested using this approach.

### RESULTS

Enrichment of viral particles had a detrimental effect on the absolute number of viral target reads, in contrast to the higher percentage of viral reads, and thus the detection limit. Internal controls, spiked EAV and PHHV, were detected with NGS. In terms of sensitivity, metagenomic NGS correlated with RT-PCR assay and resulted in additional viral pathogen detection.

### CONCLUSIONS

Optimal sample preparation is essential for metagenomics NGS based virus detection. Using a high-throughput genome extraction method without viral enrichment, we showed that sensitivity of NGS is similar to real-time PCR and even higher when encountering additionally detected viral pathogens. Although hurdles still exist, our findings provide the further foundation of moving this promising field forward for routine detection of pathogens.

## O3

**DETECTION OF REASSORTANT INFLUENZA B STRAINS FROM 2004 TO 2015 SEASONS IN BARCELONA (SPAIN) BY WHOLE GENOME SEQUENCING (WGS)**

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**BACKGROUND-AIM**

Influenza B viruses (FLUBV) have segmented genomes which enables the virus to evolve by segment reassortment. Since the divergence of both FLUBV lineages, B/Victoria/2/87 and B/Yamagata/16/88, PB2, PB1 and HA have kept the same ancestor, while some reassortment events in the other segments have been reported worldwide. The aim of the present study was to find out reassortment episodes in FLUBV strains detected in cases attended at Vall d'hebron University Hospital and Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) from 2004 to 2015 seasons.

**METHODS**

From October 2004 to May 2015, respiratory tract specimens were received from patients with RTI suspicion. Influenza detection was carried out by either cell culture isolation, immunofluorescence or PCR-based assays. A RT-PCR was performed to distinguish both lineages by agarose gel electrophoresis. Whole genome amplification was performed using the universal primer set by Zhou et al on 2012, and subsequently sequenced using Roche 454 GS Junior platform. Bioinformatic analysis was performed to characterise the sequences with B/Malaysia/2506/2007 and B/Florida/4/2006 corresponding sequences as reference, respectively.

**RESULTS**

A total of 118 FLUBV (75 B/VIC and 43 B/YAM), from 2004-2006, 2008-2011 and 2012-2015 seasons, were studied. Whole genome was successfully amplified from 54 B/VIC and 42 B/YAM viruses. Based on HA sequences, most B/VIC viruses (42; 80%) belonged to clade 1A (B/Brisbane/60/2008) except 11 (20%), which fell within clade 1B (B/HongKong/514/2009). Nine (20%) B/YAM viruses belonged to clade 2 (B/Massachusetts/02/2012), 18 (42%) to clade 3 (B/Phuket/3073/2013) and 15 (38%) fell within Florida/4/2006. Numerous intra-lineage reassortments in PB2, PB1, NA and NS were found in 2 2010-2011 viruses. Inter-lineage NP reassortment were found in all (12) 2014-2015 B/YAM viruses and in 6 2012-2013 B/YAM viruses, in addition to 1 NS reassortant among 2010-2011 B/VIC viruses.

**CONCLUSIONS**

Intra- and inter-lineage reassortment episodes were revealed by WGS. While PB2-PB1-HA remained in complex, NP and NS reassortant viruses were found in both lineages. Despite reassortment events are not often, the characterisation only by HA and NA sequences might be underestimating their detection.

## O4

**ACUTE FLACCID MYELITIS CASES PERSISTENTLY ASSOCIATED WITH SPIKES IN RESPIRATORY ENTEROVIRUS D68: CASE SERIES FROM A SINGLE PEDIATRIC MEDICAL CENTRE**

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**BACKGROUND-AIM**

In 2014, a global outbreak of Enterovirus D68 (EV-D68) caused severe respiratory disease and was associated with an increase in acute flaccid myelitis (AFM) cases. Despite increased surveillance, both EV-D68 detection and AFM reporting dropped in 2015. With increases in AFM again in 2016, we sought to better understand the epidemiology at our institution.

**METHODS**

Chart review of clinical presentation and workup was conducted on patients meeting the case definition for AFM between 2015-2016. To determine EV-D68 prevalence at CHLA, patients positive for Rhinovirus/Enterovirus (RV/EV) by FilmArray respiratory panel in September 2016 were screened for EV-D68 by RT-PCR. Results were compared to a research algorithm developed within the FilmArray Trend software. Once accurate prediction of EV-D68 was established, the algorithm was used to measure EV-D68 prevalence at CHLA in 2015 and 2016.

**RESULTS**

7 patients with no significant past medical history presented with AFM between July 15 -October 15, 2016, while none were identified in 2015. Median age was 3.3 years. All presented with acute onset upper extremity weakness and grey matter involvement on MRI. 6/7 reported fever/upper respiratory infection prior to AFM onset. CSF from 7/7 was negative by FilmArray meningitis/encephalitis panel and 2/7 were positive for EBV DNA. Further work up on CSF and blood were negative. 4/7 (57.1%) patients were RV/EV positive from respiratory samples and 3 were confirmed as EV-D68 by RT-PCR. IVIG was given in 7/7 cases. Patients were discharged after an average of 8.8 (4.8-13.6) days. The algorithm from FilmArray Trend monitoring revealed that during the time of AFM presentation in 2016, 226/778 patients tested for respiratory viruses by the FilmArray respiratory panel were positive for RV/EV. Of those, 29.2% (66/226) were positive for EV-D68 compared to 0.02% (2/224) over the same period in 2015.

**CONCLUSIONS**

As shown by CDC surveillance data, we saw a resurgence of AFM cases in 2016 compared to 2015. All 7 patients identified were previously healthy and had persistent weakness at discharge. Cases were accompanied by increases in circulating respiratory EV-D68. Further investigation of the correlation between EV-D68 resurgence and AFM is warranted.

## O5

**GLOBAL DISTRIBUTION OF HUMAN PROTOPARVOVIRUSES**

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**BACKGROUND-AIM**

The three protoparvoviruses, bufavirus (BuV), tusavirus (TuV) and cutavirus (CuV) are the newest human parvoviruses described: all were originally discovered in feces of diarrheic children - BuV in 2012 in Burkina Faso, TuV in 2014 in Tunisia and CuV in 2016 in Brazil and Botswana. To date, BuV DNA has been detected in one nasal swab and at low prevalence of <4% in feces of diarrheic patients in Africa, Europe and Asia, whereas TuV DNA has been found only in the feces of one child. CuV has, besides in feces, been detected exclusively in cancerous skin tissues of 4/17 French patients with cutaneous T-cell lymphoma and in one Danish patient with melanoma. In all, the etiological roles of these viruses in human disease remain uncertain.

**METHODS**

In our previous study, we found a low BuV seroprevalence (3.1%) in Finland, whereas 5/12 Asian staff members were BuV-IgG positive. We have since included CuV in our IgG EIA panel, and studied the global spread and possible contribution of human-animal contact. We also analyzed 137 skin samples from organ transplant patients with BuV-TuV-CuV-qPCR, as well as the corresponding serum samples (n=124) with the BuV-TuV-CuV-IgG EIA panel.

**RESULTS**

We found a striking difference in BuV geographical seroprevalence, from 2.8% among 324 Finnish veterinarians to 84.8%, 59.6% and 74.3% among 99 Iraqi and 89 Iranian healthy adults, and 105 Kenyan febrile adults, respectively. In contrast, the CuV IgG seroprevalences in all 4 countries were low (1-5%) and TuV IgG was absent. In skin four transplant patients (2.9%) harbored CuV DNA: three in healthy skin and one in both healthy skin and in squamous cell carcinoma. No BuV or TuV DNA was found.

Serum samples were available from two of the CuV-DNA-positive patients and both showed high CuV-IgG titres. The overall seroprevalence in the transplant cohort was 2.4% for BuV and 6.5% for CuV and TuV IgG was present in one (0.8%) patient.

**CONCLUSIONS**

These are the first studies on the global seroprevalence of human protoparvoviruses. BuV infections were found to be very common in the Middle East and Africa, contrary to Finland, while CuV infections were evenly distributed yet infrequent in all regions. TuV findings were rare. We further detected, for the first time, 2 CuV seropositive subjects with CuV DNA in healthy skin.

O6

# **NOVEL LINEAGES OF COXSACKIEVIRUS A06 ASSOCIATED WITH ATYPICAL OF HAND- FOOT- AND MOUTH DISEASE AND DECREASED SENSITIVITY OF VP1 TYPING PRIMERS, DENMARK 2005-2017**

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## **BACKGROUND-AIM**

Since 2008, coxsackievirus A06 (CVA-06) has been recognized as one of the main causes of Hand- Foot- and Mouth disease (HFMD). In more recent years a genetic shift in circulating strains has been seen, and the new CV-A06 strains are clinically associated with atypical HFMD. Since March 2014, when an out-of-season peak of CVA-06 detection prompted us to initiate a retro- and prospective study of CVA-06, circulation of CVA-06 has continued at a high level. In this study we present CVA-06 typing data from 12½ years of molecular enterovirus surveillance in Denmark.

## **METHODS**

Denmark has no specific surveillance for HFMD, and cases are mainly detected through the enterovirus surveillance program at the National WHO Reference Laboratory for Poliovirus (NRL) at Statens Serum Institut (SSI). SSI has been characterizing enteroviruses by molecular methods since 2005. Two regions, VP1 and/or VP2, are used for typing with RT-PCR and subsequent sequencing. Phylogenetic analysis of sequences is carried out using MEGA6.

## **RESULTS**

From January 1st 2005 until May 31st 2017, 372 samples from 322 patients were genotyped CVA-06 among 3057 enterovirus positive cases. CVA-06 was detected all year round. Prior to 2012, CVA-06 constituted ≤5% of all enteroviruses characterized at the NRL, rising to 12% in 2012, 29% in 2015, and to date 55% of cases in 2017 have been CVA-06. Phylogenetic analysis revealed several new CVA-06 lineages introduced into the Danish population, with a major shift from 2012 onwards. A comparison of VP2 vs. VP1 PCR-based typing assays showed that a major proportion (49%) of 372 CV-A06 strains are typeable by VP2 PCR only.

## **CONCLUSIONS**

The prevalence of CVA-06 HFMD appears to have increased over the past 12 years. Several novel, genetically distinct, lineages of CVA-06 have circulated in Denmark since 2012, corresponding to the increased CVA-06 prevalence. Our results show that the sensitivity of the most commonly used VP1 typing primers towards CVA-06 is lower than the VP2 primers, thereby demonstrating the benefit of targeting more than one genetic region when characterizing enteroviruses from clinical samples.

O7

# **MULTIPLE MEASLES OUTBREAKS SUSTAINED BY DIFFERENT GENOTYPES IN LOMBARDY REGION DURING AN ONGOING EPIDEMIC INVOLVING ITALY SINCE JANUARY 2017**

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## **BACKGROUND-AIM**

since the beginning of 2017 a large measles epidemic is involving Italy, as a Regional Reference Laboratory for measles surveillance in Lombardy Region (Northern Italy) we evaluated a great part of measles suspected cases. We report the identification, the diagnosis of the cases and the subsequent investigations.

## **METHODS**

serum samples of patients with potential Measles infections were tested for the presence of specific IgM and IgG antibodies (LIAISON® Measles IgM and LIAISON® Measles IgG by DiaSorin S.p.A., Saluggia, Italy). Saliva and urine, collected during the acute phase, were examined for the presence of Measles RNA with a specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR) targeting a portion of gene N (nt:584-697). The positive samples were amplified with a Nested RT-PCR targeting a portion of C-terminal end of the nucleoprotein gene and sequenced to genotype the virus.

## **RESULTS**

In the period 2 January-18 May 2017, as a Regional Reference Laboratory for measles surveillance in Lombardy Region (10 million inhabitants), Northern Italy, we evaluated 223 suspected measles cases during a large ongoing measles epidemic involving Italy since the beginning of 2017. 167/223 (75%) were confirmed measles cases by serological and/or molecular methods while 56/223 (25%) were negative. 79 PCR-measles positive sample were sequenced and the sequence analysis identified the co-circulation of genotype D8 (55/79, 70%), B3 (12/79, 15%) and H1 (12/79, 15%). All the 12 measles genotype H1 originated from an imported case, an Italian traveler returning in Italy after a vacation in Myanmar and Thailand, and involved a total of 15 cases in 2 provinces in Lombardy.

## **CONCLUSIONS**

The cases of measles detected in our Laboratory are a part of a large ongoing national epidemic involving 2395 cases from 1 January to 14 May 2017.

O8

**HEPATITIS E VIRUS EMERGENCE IN NORTH EASTERN FRANCE**

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**BACKGROUND-AIM**

Hepatitis E is an emerging disease including hepatitis and extra-hepatic signs in human beings. Hepatitis E virus (HEV) is a food- and water- transmitted virus. However, HEV circulation between humans and the environment remains misunderstood. Our aim was to explore by molecular tools HEV transmission between patients and the environment in North-Eastern France. We studied HEV molecular characteristics in ORF2 gene, the viral capsid playing a key role in viral interactions with various surfaces, cellular membranes and host-related immunity.

**METHODS**

Samples were collected from patients with hepatitis (University Hospital, Nancy; n=85, blood and stools), from the entrance of a waste water treatment plant (WWTP; n=9), a pig slaughterhouse (n=11), from pigs (n=15 stools) and wild boars (n=39 liver and stool samples). After HEV RNA quantification by RT-qPCR, viral sequences from direct and ultra-deep sequencing (UDS, MiSeq/Illumina) were analyzed within ORF2 gene by bioinformatics (MEGA/ Geneious/AnTheProt) for genetic variability, phylogeny and physicochemical prediction.

**RESULTS**

HEV was detected in six patients (six blood, three stool samples), three WWTP samples, nine samples from the slaughterhouse, two wild boars (two liver, one stool sample), at highly variable quantitation levels. After direct sequencing and UDS for six patients and one boar liver, a phylogenetic tree classified the strains as HEV genotype 3. HEV sequences obtained by UDS highlighted the same homogeneous major viral variant in all quasispecies, above 85% homology at the amino acid level. Thus, in spite of rather variable nucleic acids, a low variability in amino acid residues was observed; only two minor mutations, D442G (two patients) and V402A (one patient) led to a decrease in predicted antigenicity.

**CONCLUSIONS**

HEV was highlighted by RT-qPCR in patients, in environmental samples collected at the entrance of a WWTP, from a pig slaughterhouse and in wild boars. Even though variable nucleic acid sequences were detected in ORF2, a low variability of amino acids was observed allowing us to assume that homogeneous HEV strains could be advantaged for structural and biological properties of the capsid and circulate as dominant viruses in North-Eastern France.

O9

**IMMUNOSUPPRESSION-DRIVEN HBV REACTIVATION CORRELATES WITH A RELEVANT RISK OF DEATH AND WITH EVOLUTION TOWARDS ACTIVE CHRONIC INFECTION**

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**BACKGROUND-AIM**

This study presents virological and clinical features of a cohort of patients developing immune-suppression driven HBV-reactivation.

**METHODS**

80 consecutive-patients with HBV-reactivation were included (66.2% treated with rituximab, 10% with corticosteroids, and 23.8% with other chemotherapeutic-agents). Patients were monitored for a median of 30(IQR:14-46) months from reactivation-diagnosis.

**RESULTS**

At HBV-reactivation diagnosis (median[IQR]time:2012[2011-2013]), median HBV-DNA and ALT were 6.7(IQR:4.5-7.9) logIU/ml and 117(IQR:40-621)U/L; 8.9% of patients died for hepatic-failure.

HBV serological profiles before immunosuppressive-therapy showed a large fraction of HBsAg-negative patients (isolated anti-HBc:37.5%, anti-HBc/anti-HBs:17.5%, isolated anti-HBs:5%), followed by inactive-carriers (21.3%) and active-carriers (2.5%). 3.7% resulted negative to all HBV-markers, and 12.5% was not screened for HBV-markers.

17.6% of anti-HBc-positive/HBsAg-negative patients remained HBsAg-negative at HBV-reactivation (despite high viremia). In 71.4% of them, an additional N-linked glycosylation site was detected in HBsAg (decreasing recognition by neutralizing- and diagnostic-antibodies).

30% of HBV-reactivated patients were under lamivudine-prophylaxis (median duration:22[IQR:14-30]months). Among them, 55% of patients experienced HBV-reactivation during lamivudine-administration (drug-resistance mutations detected in 36.4% of cases). The remaining 45% (mainly rituximab-treated) experienced HBV-reactivation after stopping lamivudine, suggesting incongruous prophylaxis duration despite profound immunosuppression.

Among HBsAg-negative patients at HBV-screening, after

starting antiviral-treatment for HBV-reactivation, 68.6% of them remained HBsAg-positive despite potent antiviral-treatment (median treatment-duration:3.5 years), with consequent transformation of silent infection into chronic HBV-hepatitis.

#### CONCLUSIONS

This study (the largest so far conducted) shows the need of a joint-effort to improve the management of HBV-reactivation in terms of adequate screening, monitoring and prophylaxis in all clinical-settings requiring the use of immune-suppressive agents (including those today perceived at lower reactivation-risk).

#### O10

#### A BI-CENTRIC LONGITUDINAL ANALYSIS OF HCMV-SPECIFIC T-CELL IMMUNE RESPONSE AMONG KIDNEY TRANSPLANT RECIPIENTS (KTRs)

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#### BACKGROUND-AIM

Human Cytomegalovirus (HCMV) is one of the most relevant viral infection among solid organ transplant recipients. The aim of this bi-centric prospective study was to analyze HCMV-specific T-cell response in kidney transplant recipients (KTRs) in relation to HCMV reactivation and the onset of related-diseases.

#### METHODS

44 KTRs [33 males (75%) and 11 females (25%)] were recruited at IRCCS Policlinic San Matteo (Pavia) and "Città della Salute e della Scienza" Hospital (Turin). HCMV serostatus was assessed at pre-transplant and HCMV DNAemia was weekly monitored after transplant. Peripheral blood mononuclear cells (PBMC) were collected at 0, 30, 60, 90, 180, 270 and 360 days after transplant. Lymphocytes were stimulated with peptide pools (15 aminoacids in length with 11 overlapping) representative of the whole HCMV protein pp65, IE1 and IE2. IFN producing T cells were quantified by ELISpot assay (Elitech group, Italy) as net spots/million PBMC. Responses were normalized on positive control (PHA) and then on CD4+ and CD8+ T-cell counts.

#### RESULTS

Two HCMV seronegative patients (4,5%) received the organ from seropositive donor (D+/R-), developing HCMV primary infection, while the other 42 (95,5%) were HCMV seropositive at transplant. Analysis of HCMV protein-specific T-cell responses at pre-transplant showed that pp65 was the most immunogenic antigen, followed by IE1; IE2-specific T-cell response was almost undetectable. According to HCMV DNAemia, KTR were classified in two groups (viral load  $\geq 100.000$  copies/mL; n=12 and viral load  $< 100.000$  copies/mL; n=32). We observed a significantly lower IE1-specific CD4+ T-cell response among the first group of KTR (median 0.01 IQR 0.0-0.04 CD4+ T-cells/ $\mu$ l) respect to patients with viral load  $< 100.000$  copies/mL (median 0.05 IQR 0.01-0.24 CD4+ T-cells/ $\mu$ l), p= 0.0349. A preliminary longitudinal analysis showed that both CD4+ and CD8+ HCMV-specific T-cell responses were lower among patients with high risk HCMV infection during the first 6 months after transplant.

#### CONCLUSIONS

Evaluation of HCMV-specific T-cell response at pre-transplant is useful to predict the development of HCMV infections at risk for disease. The role of IE1-specific T-cell response as a prognostic predictor requires confirm in a larger group of patients.

O11

### NEXT GENERATION SEQUENCING FOR THE DETECTION OF ANTIVIRAL DRUG RESISTANT HERPES SIMPLEX VIRUS TYPE 1 SUBPOPULATIONS IN IMMUNOCOMPROMISED PATIENTS

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#### BACKGROUND-AIM

Herpes simplex viruses (HSV) are a major cause of morbidity in immunocompromised patients. Its high replication, not controlled by immune system favors the emergence of strains resistant to antiviral drugs. Accurate diagnosis for drug resistance is important to optimize their clinical management. Sanger sequencing remains largely used for targeting UL23 and UL30 genes, in order to detect resistance mutations but cannot detect precisely viral subpopulations.

**Aim:** This retrospective study developed an optimized NGS sequencing for HSV-1, never published before. Using the NextSeq 500 Illumina® sequencer, this study aimed to show the “hidden part” of the viral population to understand the kinetic of the viral switch between sensitive and resistant viral subpopulations. These data altogether give the ability to follow precisely mutations present in UL23 and UL30 genes, in order to adapt, in a short delay, antiviral treatment.

#### METHODS

Five immunocompromised patients with antiviral drug resistant HSV-1 reactivations were followed between 2012 to 2016. Four to ten samples per patient were used to analyze the hidden part of viral subpopulations using NextSeq 500® platform (Illumina, San Diego, USA). NGS performances were quantified for the detection of 4 different types of mutants: 3 UL23 mutants presenting either an addition or a deletion or a substitution and one UL30 substitution mutant.

#### RESULTS

With an optimized NGS protocol and using viral mock subpopulations, threshold was set to 2% of the minor subpopulation. In patients, main mutations responsible for drug resistance were followed, in all sequenced samples. Unexpected polymorphisms could be detected at low frequency in both genes. Interestingly, other UL23 gene resistance mutations than the major and expected one were also found and stayed in low percentage in the viral subpopulation (<5%). Sequencing data were correlated to clinical metadata.

#### CONCLUSIONS

NGS for the detection of viral subpopulations of HSV-1 in immunocompromised patients showed that viral subpopulations are patient-specific. NGS methods for the early detection of antiviral-resistant HSV-infected patients could key in clinical routine management of immunosuppressed patients.

O12

### ASSOCIATION OF TORQUE TENO VIRUS LOAD WITH THE EFFICACY OF IMMUNOSUPPRESSION AFTER LUNG TRANSPLANTATION

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#### BACKGROUND-AIM

Monitoring of immunosuppression after organ transplantation (TX) is still a challenge. First studies have shown that the Torque-Teno Virus (TTV) DNA load in blood may provide a marker for the level of immunosuppression in lung transplant recipients (LTRs). Therefore a prospective study was performed to investigate whether TTV-load levels predict development of microbial infections or episodes of organ rejection in the post-TX follow up.

#### METHODS

In this study 143 LTRs were prospectively followed up for 235-1416 days post TX and within the follow up 2675 plasma samples (at least 10/patient) were tested by quantitative TTV-DNA PCR. Patients were grouped based on previous data to a TTV-high (>9.5 log10 copies/ml TTV-DNA for at least 30 days), a TTV-low (<7 log10 copies/ml TTV-DNA for at least 30 days), and a TTV-ideal group (none of the above). The data were correlated to further development of microbial infections and episodes of chronic lung allograft dysfunction (CLAD).

#### RESULTS

Within the first year post TX 26 patients developed microbial infections. The incidence of infection was significantly higher in patients of the TTV-high group (24/54 patients, 44%) than in those of the ideal (2/77; 2,6%) and the TTV-low (0/12) group, respectively (Kaplan Meier: p<0.001 and p=0.015). No CLAD was observed. A total of 124 patients were further followed up for the second year post TX. CLAD was observed in 20 patients, and was significantly higher in patients who were in the TTV-low group in the second year (15/38 patients; 39,5%) than in those of the ideal (4/72, 5,6%) and the TTV-high (1/14; 7,1%) group, respectively (Kaplan Meier: p=0.002 and p=0.018). Five infections were observed in the second year, all in the TTV-high group.

#### CONCLUSIONS

The TTV-load in blood may be used as a marker for the level of immunosuppression in the first years after lung TX and may predict microbial infections and episodes of CLAD.

O13

### DIVERSITY OF HEPATITIS B SURFACE ANTIGEN VARIANTS AMONG ACUTE HEPATITIS B VIRUS INFECTIONS IN THE NETHERLANDS

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#### BACKGROUND-AIM

Substitutions within HBsAg have been related to immune/vaccine and test failures and can have a significant impact on vaccination and diagnosis of acute infection. The aim of this study was to investigate the frequency and diversity of the Hepatitis B surface antigen (HBsAg), in particular within the Major Hydrophobic Region (MHR).

#### METHODS

All acute primary HBV infections anonymously notified and chronic HBV infections to OSIRIS by the municipal health services and typed between 2004 and 2014 were studied. The amino acid sequence variation was by aligning all sequences using Bionumerics version 7.1 to the reference sequences assigned by Pourkarim MR, 2014.

#### RESULTS

HBV that was isolated from 1232 cases and the S gene was sequenced. HBsAg amino acid substitutions were analyzed by genotype to assess amino acid diversity. Analysis of the HBsAg revealed the circulation of 6 genotypes (Gt); GtA was the dominant genotype (68.1%) followed by GtD (17.4%). However, the frequency and diversity of GtD variants was significantly higher than for GtA ( $p < 0.001$ ). GtD also contained the most MHR-substitutions (6.4%). Two thirds of GtD-MHR variants carried T118V/P127T, which were also identified in variants isolated from 2 fully vaccinated cases.

#### CONCLUSIONS

In conclusion, we show a high frequency and diversity of the HBsAg among Dutch acute HBV infected cases, in particular among those infected with GtD. The high frequency and diversity of GtD most likely represent transmission from (unknown) chronically infected immigrants.

O14

### GASTROINTESTINAL CELLS REPRESENT AN EXTRAHEPATIC RESERVOIR OF HCV: POSSIBLE ROLE IN HCV NATURAL HISTORY AND LIVER REINFECTION AFTER TRANSPLANTATION

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#### BACKGROUND-AIM

Hepatitis C virus (HCV) mainly infects hepatocytes, despite its receptors are expressed by many cell types. Several extrahepatic (EH) manifestations characterize the HCV infection suggesting a viral tropism to EH tissues that has to be completely demonstrated. In this study we investigated the gastrointestinal mucosa (GIM) as EH site of HCV replication and its role in HCV recurrence after liver transplant (LT).

#### METHODS

We analyzed GIM biopsies from 76 (11 HCV- and 65 HCV+) patients, whose 54 underwent to LT. In 29 cases we examined biopsies pre- and post-OLT from each patient. To evaluate HCV infection in GIM biopsies, we looked for the presence of viral proteins by immunohistochemistry and of total HCV RNA by RT-PCR. We also assessed the presence of the replicating virus by the detection of the minus-strand HCV RNA and we identify the preferably cell type infected by the virus by Ag co-localization imaging. Moreover, we conducted a phylogenetic analysis of the HCV quasispecies from liver, plasma and GIM to investigate the viral compartmentalization and the possible contribution of the GIM HCV variants to liver reinfection after LT.

#### RESULTS

GIM resulted positive for viral RNA and proteins demonstrating that HCV can infect this tissue. The presence of the minus-strand HCV demonstrated the ability of the virus to replicate in GIM. Our results showed that HCV infects mainly enteroendocrine cells (EEC) and alters their functionality by increasing the somatostatin levels. By Mantel's test we observed a compartmentalization of the HCV variants detected in GIM compared to those present in the other analyzed tissues of the same patient. Moreover, the phylogenetic analysis showed a similarity between the HCV quasispecies from GIM before LT and that from liver after LT, for the same patient.

#### CONCLUSIONS

HCV was founded replicate and evolve independently in GIM, for this reason this tissue might be considered an EH reservoir of the virus and might have a role in viral persistence. We found

EEC as the main target of HCV. Because of the multifunctional role of this cell type, mostly involved in regulation of the immune system during infective and inflammatory disease, further studies could help to clarify their role in the natural history of HCV infection.

## O15

### HEPATITIS DELTA ANTIGEN IS CHARACTERIZED BY AN EXTENSIVE DEGREE OF GENETIC VARIABILITY THAT CORRELATES WITH ELEVATED LEVELS OF SERUM HDV-RNA

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#### BACKGROUND-AIM

HDV-antigen (HDAg) interacts with HBV surface protein (HBsAg). No information is available on the extent of genetic variability in HDAg and its impact on virological parameters.

#### METHODS

Among 78 patients (pts) with chronic HBV+HDV infection, HDAg gen-1 sequences are obtained for 47 pts and HBsAg gen-D sequences for 31 pts.

Shannon Entropy (SE) is used to measure the extent of amino acid variability at each HDAg- and HBsAg-position in overall population and by stratifying patients according to serum HDV-RNA: 18 pts with HDV-RNA<5logIU/ml defined as low-viremic and 29 with HDV-RNA>5logIU/ml defined as highly-viremic. Positions with SE=0 are defined conserved.

#### RESULTS

A lower % of conserved residues is observed in HDAg than HBsAg (49.5% vs 69.2%,  $P<0.001$ ). In particular, HDAg-domains with a lower % of conserved positions are multimerization-domain (MD, aa:31-52) and RNA-binding domains (RDBs, aa:2-27; 97-107; 136-146), followed by nuclear-localization signal (NLS, aa:68-88) and viral-assembly signal (VAS, aa:195-214) (% conserved residues: 27.3%, 31.3%, 52.4% and 70%, respectively).

Stratifying pts according to HDV-RNA, a higher degree of genetic variability is observed in highly-viremic than in low-viremic pts (%conserved residues: 55.6% vs 65.9%,  $P=0.037$ ). In addition, HDAg-mutations S6R, A22S and L90S significantly occur more frequently in highly-viremic than in low-viremic pts (S6R: 24.1%[7/29] vs 0%[0/18],  $P=0.034$ ; A22S: 72.4%[21/29] vs 33.3%[6/18],  $P=0.015$ ; L90S: 31%[9/29] vs 0%[0/18],  $P=0.008$ ). In particular, median(IQR) serum HDV-RNA in presence of >1 of these mutations is 5.7(5.1-6.3) vs 4.4(3.8-5.6) in their absence ( $P=0.003$ ). S6R and A22S reside in RDB-I, while L90S is close to NLS (domains crucial for HDV life-cycle), suggesting that these mutations can confer a replicative advantage to HDV.

#### CONCLUSIONS

An extensive genetic variability in HDAg correlates with elevated serum HDV-RNA, suggesting a still ongoing evolutionary HDV adaptation to human host. This genetic variability should be taken into account for the design of novel pharmacological targets.

## O16

**HCV ERADICATION STILL DIFFICULT FOR GENOTYPE 4R**

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**BACKGROUND-AIM**

HCV genotype 4 represents 13-20% of infections worldwide and is highly diverse with marked geographic differences in subtypes distribution. The purpose of this study was to assess the prevalence of resistance associated substitutions (RAS) of G4 to sofosbuvir or NS5A inhibitors at baseline and their impact on sustained virological response (SVR) to Direct-acting antivirals (DAAs)..

**METHODS**

From 2014 to 2016, 91 G4-infected patients were treated with interferon-free DAAs (G4 w/o assigned subtype 30.8%, G4a 28.6%, G4d 17.6%, G4r 5.5%, G4f 3.2%, G4h 3.2%, G4k 2.1%, G4m 1%, G4n 4.3%, G4o 1%, G4t 1%, G4v 1%. SVR12 was assessed and resistance testing was performed at baseline and at relapse in NS3 (position 1-180), NS5A (1-100) and NS5B (1-320). Additional NS5A and NS5B sequences were obtained from 31 DAAs naïve G4r-infected patients to assess the prevalence of RAS.

**RESULTS**

Among DAAs naïve or baseline G4r-infected patients, NS5A RAS prevalence was 95%, 100%, 80%, 8.3%, and 91.6% at positions 28 (28V/T/M/F/T/I), 30 (30R), 31 (31L), 58 (58S) and 62 (62D/H/N/S/T), respectively; and the prevalence of NS5B C316H and V321I RAS was 26%.

SVR was obtained in 93% of G4-infected patients. Virological failure (VF) was observed in 1/26 (4%), 1/16 (6%) and 5/5 (100%) G4a, G4d, and G4r-infected-patients, respectively. 2/5 G4r-infected patients treated with paritaprevir+ombitasvir+RBV, presented baseline NS5A RAS at positions 28,30, 31, and 62, and 1 developed NS3 168A at VF; 1 patient treated with ledipasvir+sofosbuvir had NS5A RAS at positions 28,30 and 62 associated with baseline NS5B C316H and V321I both at baseline and VF; 1 simeprevir+sofosbuvir+RBV-treated patient had baseline NS5B C316H+V321I with wild type NS3 at baseline and VF; the last one treated with sofosbuvir+RBV had wild type NS5B.

**CONCLUSIONS**

Commercially available reagents are not able to assign G4r subtype. Accurate G4r subtype assignment is only obtained by sequencing. Our results confirm the high frequency of baseline NS5A polymorphisms that may impact SVR. We also identified in G4r sequences the NS5B C316H and V321I substitutions previously reported to be associated with sofosbuvir resistance for other genotypes. Our results also suggest a potential impact of these NS5B substitutions on G4r response to sofosbuvir-based regimens.

## O17

**RETROVIRUSES; POST-TRANSCRIPTION RESTRICTION AND MICRORNAS**

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**BACKGROUND-AIM**

The xenotransplantation of encapsulated porcine islet cells has been demonstrated to be an effective treatment for type 1 diabetes in countries such as New Zealand, Russia and Argentina. In Europe this treatment has yet to be approved with one of the barriers to its clinical application being the risk posed by Porcine Endogenous Retrovirus (PERV) zoonosis. To examine the risk of PERV zoonosis the use of porcine islets poses, we investigated the expression of PERV in porcine islets.

**METHODS**

PERV RNA was detected by qRT-PCR targeting PERV pol, RT activity by SG-PERT, PERV protein by western blot. APOBEC3 and Tetherin gene expression was measured by qRT-PCR. Sucrose gradients were used to assess ribosome attachment. MicroRNA prediction was performed using RNAhybrid and RegRNA2.0.

**RESULTS**

It was found that porcine islets express PERV RNA with a mean copy number of 8839±873 copies per 250ng RNA (n=80), in comparison to a mean of 4526±1746 copies per 250ng in corresponding PBMCs. However, no PERV release could be detected in the culture medium of porcine islets, either by assaying for viral RNA or enzymes (n=16). In addition, PERV protein could not be detected in porcine islet cells, although it was in porcine PBMCs (n=12), suggesting post transcriptional inhibition of PERV protein expression occurs within porcine islet cells. Known host mechanisms of PERV post transcriptional restriction mechanisms, include cytidine deamination by APOBEC3 proteins and diffusion prevention by Tetherin. However no increased expression in APOBEC3 proteins or Tetherin could be detected in islets in comparison to PBMCs (n=8).

**CONCLUSIONS**

We concluded an alternative mechanism of post transcriptional inhibition of PERV occurs in porcine islets, possibly the expression of a microRNA (miRNA) which prevents PERV-ribosome attachment. Several putative PERV targeting miRNAs have been identified. Of particular interest are ssc-miR-204, ssc-miR-396-5p, ssc-let-7c and ssc-miR-190b, as the human homologues of these are known to be upregulated in human islet cells. In vitro analysis of putative PERV acting miRNAs in islet cells, followed by expression vector experiments of these miRNAs to demonstrate their effect on PERV expression and possible cross over to human retroviruses is ongoing.

O18

**RED BLOOD CELLS ARE A NATURAL SLOW RELEASE DEPOT FOR ELSULFAVIRINE/VM1500A HIV NNRTI**V. Bichko<sup>1</sup><sup>1</sup>Viriom, Inc.**BACKGROUND-AIM**

Elsulfavirine (Elpida®, VM1500) is the prodrug of VM1500A, a new, potent non-nucleoside reverse transcriptase inhibitor (NNRTI), currently under review for registration as an oral QD regimen for HIV/AIDS treatment. Unique pharmacokinetic properties (T<sub>1/2</sub> ~8 days) of VM1500A suggest a possibility for long-acting formulation development (Ratanasuwan et. al, IAS 2014, Abstract LBPE20). A proof-of-concept animal study on Elsulfavirine long acting injectable (LAI) formulations was recently reported (Bichko et. al, IAS 2017, Abstract TBD). The reason for Elsulfavirine long T<sub>1/2</sub> remains unclear. VM1500A contains a sulfonamide moiety, which has a potential for binding to red blood cell (RBC) carbonic anhydrase. The aim of this study was to test the hypothesis that VM1500A accumulates in RBCs via reversible binding to RBC carbonic anhydrase, and its slow release back to plasma explains its prolong half-elimination time.

**METHODS**

Rats and dogs received a single oral Elsulfavirine dose (500 and 10 mg/kg, respectively), and the blood samples were drawn 3 hours post-dose. In the clinical studies, healthy volunteers received oral Elsulfavirine doses of 10, 20 or 30 mg QD for 14 days. Elsulfavirine and VM1500A in all blood, blood cells and plasma samples were analyzed using a liquid chromatography-tandem mass spectrometry method (LC-MS/MS).

**RESULTS**

VM1500A was extensively partitioned into blood cells after oral dosing of Elsulfavirine to rats and dogs. The blood-to-plasma ratio was similar across species (7.5 for rats and 11 for dogs). Only sulfonamide-containing metabolites of VM1500A were extensively partitioned to blood cells. There was no accumulation of Elsulfavirine in blood cells. Similar observation was made in the clinical studies. In healthy subjects, the levels of VM1500A were significantly higher in blood cells as compared to plasma (C<sub>max</sub> cells/plasma ratio of 6.6 – 11 (depending on dose)).

**CONCLUSIONS**

Upon oral administration, Elsulfavirine is quickly converted to VM1500A that reversibly accumulates in RBCs. That allows drug to be released back to plasma. Thus, RBCs serve as a natural slow release depot for VM1500A, leading to a very slow elimination of the drug from plasma.

O19

**ANALYSIS OF THE ASSOCIATION OF HIV-1 LOW LEVEL VIREMIA AND VIROLOGICAL FAILURE TO ANTIRETROVIRAL THERAPY**N. Lübke<sup>5</sup>, A. Pironti<sup>3</sup>, E. Knops<sup>6</sup>, E. Heger<sup>6</sup>, B. Jensen<sup>4</sup>, M. Oette<sup>1</sup>, S. Esser<sup>2</sup>, T. Lengauer<sup>3</sup>, J. Timm<sup>5</sup>, R. Kaiser<sup>6</sup><sup>1</sup>Clinic for General Medicine, Gastroenterology, and Infectious Diseases, Augustinerinnen Hospital, Cologne, Germany<sup>2</sup>Clinic of Dermatology, Venerology and Proctology, University Hospital Essen, Essen, Germany<sup>3</sup>Department of Computational Biology and Applied Algorithmics, Max-Planck-Institute for Informatics, Saarbrücken, Germany<sup>4</sup>Department of Gastroenterology, Hepatology and Infectiology, University Hospital Düsseldorf, Düsseldorf, Germany<sup>5</sup>Institute of Virology, Heinrich-Heine-University, Düsseldorf, Germany<sup>6</sup>Institute of Virology, University of Cologne, Cologne, Germany**BACKGROUND-AIM**

HIV-1 Low Level Viremia (LLV) is a constant problem in the management of HIV therapy, as it has been previously shown to be predictive to virological failure (VF). Here, we provide an independent analysis of the association of LLV and subsequent VF to antiretroviral therapy (ART).

**METHODS**

We screened the German AREVIR database for patients who had confirmed therapeutic success under ART and who developed a LLV thereafter. In our study, LLV is defined as repeated Viral Load (VL) measurements between 50 and 200 copies/ml and VF as a confirmed VL greater than 200 copies/ml after initial therapeutic success. p-values were calculated with Fishers' exact and Wilcoxon rank sum test.

**RESULTS**

The database query resulted in 2,485 first-line and 3,657 further-line therapies (n=6124). LLV occurred in 294 (4.8%) of these therapies, specifically in 47 (1.9%) first-line and in 247 (6.8%) further-line therapies. The majority of patients showing LLV were treated with PI-based therapies, followed by NNRTI-based regimens (165/294; 56.1% and 76/294; 25.9%). 53 out of 294 (18%) patients experienced VF after LLV with a median VL at failure of 472 copies/ml. The failure rate was higher in further-line therapies (48/247; 19.4%), as compared to first-line treatment (5/47; 10.4%); (p=0.2129). The VF rates showed no significant difference between the drug classes (Ø=20%, range 17.1-22.2%). Therapies showing LLV and subsequent VF started on average in 2002 while those without VF in 2005 (p<0.0001). Moreover, VF was never related to entry inhibitors, integrase inhibitors or the more recently approved compounds DRV, TPV, and RPV (45/204 vs. 0/83, respectively; p<0.0001). The lowest risk for the development of resistance mutations during LLV was observed with PI-based regimens (45.5%).

**CONCLUSIONS**

The prevalence of LLV in patients on suppressive ART is low (4.8%). Nevertheless, 18% of patients with LLV developed VF thereafter. The strongest predictor for VF after LLV was therapy experience of the patients and the treatment with older drug regimens based on drugs approved before 2005. Therefore, episodes of LLV in patients treated with drugs with high potency and a high barrier to resistance are not predictive for VF.

O20

### IN VITRO AND IN VIVO INHIBITION OF THE CCL2/CCR2 AXIS INDUCES THE EXPRESSION OF THE ANTI-HIV FACTOR APOBEC3A

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#### BACKGROUND-AIM

The CCL2/CCR2 axis plays a key role in chronic inflammation in HIV+ patients. We recently showed that CCL2 neutralization by specific antibodies (Ab) in monocyte-derived macrophages (MDMs) restricts HIV replication by inhibiting viral DNA accumulation independently of SAMHD1. In this study, we performed a global gene expression analysis to identify cellular factors modulated by CCL2 blocking and potentially involved in HIV replication restriction. We also defined the effect of in vivo treatment with cenicriviroc (CVC), a CCR5/CCR2 antagonist which completed phase II trial for HIV infection therapy (Study 202; NCT01338883), on selected anti-HIV factors expression.

#### METHODS

MDMs from 3 donors were exposed to anti-CCL2 or control Ab for 4 and 20 h. Total RNA was extracted, subjected to poly(A) selection, reverse transcription, generation of cDNA libraries and sequencing on an Illumina HiSeq 2500 platform. Differential expression analysis was carried out using DESeq2. Genes with logFC $\geq$ 1 (upregulated) or logFC $\leq$ -1 (downregulated) and adjusted p-value  $<0.1$  were classified as significantly differentially expressed. Pathway analysis was done using DAVID and Gorilla. The differential expression profile of some genes was confirmed by qPCR. Whole cell extracts from MDMs, as well as from PBMCs of 32 Study 202 HIV+ participants (baseline, 4, 12, 24 and 48 weeks of treatment), were used for western blot analysis of selected factors.

#### RESULTS

CCL2 neutralization in MDMs results in the differential expression of 1915 and 311 genes at 4 and 20 h, respectively. Among the upmodulated genes annotated in categories related to immune responses, we focused on the restriction factors Mx2 and APOBEC3A (A3A), whose mechanisms of action may account for the CCL2 blocking-mediated postentry inhibition of HIV replication. The upregulation of both genes following CCL2 blocking is confirmed by qPCR, while an increase of protein level is observed only for A3A. An upregulated A3A expression is also found in Study 202 patients at 48 weeks of treatment with CVC 200 mg ( $p<0.05$  vs. baseline and vs. participants treated with conventional therapy).

#### CONCLUSIONS

Overall, these data suggest that the CCL2/CCR2 axis may represent a new therapeutic target to strengthen host innate immunity thus limiting HIV infection. RF-2011-02347224.

O21

### THE HUMAN ENDOGENOUS RETROVIRUS H IS OVER EXPRESSED AND HYPOMETHYLATED IN THE TISSUES OF COLORECTAL CANCER PATIENTS

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#### BACKGROUND-AIM

Human Endogenous Retroviruses (HERVs) are remnants of ancient exogenous retroviral infections of the humans, representing about 8% of the human genome. Several reports have shown the existence of a relationship between the HERVs expression and tumors, based on the mRNA expression profile of HERVs in normal and cancer tissues, but conclusive evidence is still lacking.

#### METHODS

The expressions of the env genomic region of HERV-H, HERV-K, HERV-P, and HERV-R were evaluated in the peripheral blood mononuclear cells (PBMCs), in the tumor and the adjacent normal tissues of 25 colorectal cancer patients. A group of control composed by PBMCs of 46 healthy subjects (HC) was also included in the study. RNA was isolated from the clinical specimens, reverse transcribed, and subjected to relative quantitative Real Time PCR. The env expressions were related to the expression of the housekeeping GAPDH gene. The quantification was conducted using comparative Ct method and the difference between the levels of env gene expression in the different specimens was given by fold difference. Fold difference values were relative to a calibrator, the PBMCs of patients first and the PBMCs of the healthy subjects, then. Methylation status of the HERV- H, -K and -P LTRs was evaluated by means of bisulfite-PCR and pyrosequencing.

#### RESULTS

Higher levels of expression of HERV-H, HERV-K and HERV-P were found in tumor tissues, as compared to adjacent normal tissues (5.1, 4.0 and 4.0 increasing folds, respectively) and PBMCs (5.7, 6.6, and 12.5 increasing folds respectively). The expressions of HERV-H, HERV-K and HERV-P env were also increased in the tumor tissues compared to the HC PBMCs (8.3, 2.3 and 4.4 folds respectively). No differences were observed in the expression of HERVs env among HC PBMCs, PBMCs and normal adjacent tissues of patients. HERV-H LTR was hypomethylated in the tumor tissues (55.8%) compared to normal adjacent tissues (73.0%) and PBMCs (86.8%). No significant differences were found in the methylation status of HERV-K and P LTRs.

#### CONCLUSIONS

Increased expression of HERV-H env in association with the demethylation of its LTR might support the hypothesis of specific liberation of HERV-H LTRs from epigenetic controls in colon cancer.

O22

### GAIN OF POSITIVELY CHARGED AMINO ACIDS AT SPECIFIC POSITIONS OF HBSAG C-TERMINUS IS TIGHTLY CORRELATED WITH HBV-INDUCED HEPATOCELLULAR CARCINOMA BY AFFECTING THE STRUCTURAL FOLDING OF THIS DOMAIN

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#### BACKGROUND-AIM

The gain of positively charged amino acids (aa) can hamper the folding of a transmembrane protein domain. HBsAg C-terminus is a hydrophobic transmembrane domain, composed by alpha-helices, critical for proper HBsAg secretion. Altered HBsAg folding in the membrane of endoplasmic reticulum (ER) can alter HBsAg secretion, a phenomenon involved in the initiation of HBV-induced hepatocarcinogenesis. No information is available on the role of mutations associated with gain of charged aa in HBsAg C-terminus on HBV-induced HCC onset.

#### METHODS

This study includes 807 HBV chronically infected patients from routine clinical practice: 28 with HCC (78.6% genotype D; 21.4% A), and 779 patients without HCC (79.8% D; 20.2% A). Hydrophobicity profiles of HBsAg C-terminus are constructed to predict stability of a domain in a membrane. I-Tasser is used to predict HBsAg structures (aa:1-226) and their stability.

#### RESULTS

The gain of >1 positively charged aa at HBsAg C-terminus positions 204, 207, and 210 tightly correlates with HCC (71.4% with HCC vs 30.2% without HCC,  $P<0.001$ ). Multivariable analysis confirms this correlation correcting for patients' demographics, HBV genotype, serum HBV-DNA and anti-HBV drugs use (OR[95%CI]:6.3[2.6-15.3],  $P<0.001$ ). The gain of positively charged aa derives from mutations S204R, S207R and S210R present in 14.3%, 28.6% and 28.6% of HCC-patients. S204R, S207R and S210R determine a reduction in hydrophobicity index of HBsAg C-terminus compared to wt (S204R:16.0, S207R:16.0, S210R:16.2 vs wt:16.4), and in G values (G[S204R-wt]=-0.27; G[S207R-wt]=-0.11; G[S210R-wt]=-0.14). Moreover, S207R and S210R determine a shortening of membrane-spanning alpha-helix motif compared to wt (predicted alpha-helix length: aa209-224 for S207R and S210R vs 205-225 for wt). This suggests an impaired HBsAg C-terminus stability in presence of these mutations.

#### CONCLUSIONS

Gain of positively charged amino acid at specific HBsAg C-terminus positions tightly correlates with HCC, by altering the proper folding of this domain in ER membrane. These mutations might affect HBsAg secretion and in turn contribute to the initiation of HBV-related tumorigenesis. Their role in identifying patients at higher HCC-risk deserves further investigation.

O23

### POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER IN PAEDIATRIC RENAL TRANSPLANT RECIPIENTS BEFORE AND AFTER TACROLIMUS IMMUNOSUPPRESSION

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#### BACKGROUND

Post-transplant lymphoproliferative disorder (PTLD) is the most common post-transplant malignancy in paediatric recipients. The majority of cases are Epstein-Barr virus (EBV) positive and B-cell in origin. Paediatric recipients are usually EBV seronegative and susceptible to acquiring primary infection from the donor organ, or through social contact. The risk of developing PTLD in solid organ transplant recipients is highest in the first year post-transplant, and the risk of lymphoma remains elevated lifelong.

#### METHODS

A retrospective analysis was carried out of paediatric renal transplant recipients at our centre who were transplanted and developed PTLD from 2000-2015. We reviewed clinical data including EBV serology, EBV viral loads (VL), histopathological diagnoses, management and outcomes. Routine serology and VL surveillance began in 2003 and 2009 respectively.

#### RESULTS

167 paediatric renal transplants were carried out at our centre from 2000-2015. In this period 9 patients developed PTLD (5.4%), all but one of whom were seronegative at the time of transplantation. Only two patients developed PTLD within 12 months of their transplant, with the average occurring after 3 years (95% CI 0.5-5.5,  $p=0.024$ ).

Three patients, transplanted prior to the introduction of frontline tacrolimus immunosuppression, developed EBV-associated polymorphic PTLD 4 to 10.8 years post-transplant (mean 6.5 years). After the introduction of tacrolimus, six patients developed PTLD, five after 6-30 months (mean 15 months) and one after 4.3 years. These five had all seroconverted and had detectable VLs 2-10 months post-transplant. Of the six PTLDs, two were classified as early PTLD, one was polymorphic, and three monomorphic B cell PTLD. All patients were effectively treated with a combination of reduction in immunosuppression, chemotherapy, rituximab and CTLs. Eight patients remain in remission and one died 7 years after diagnosis due to post-operative complications. High viral loads continue to be observed in some survivors.

#### CONCLUSION

The introduction of tacrolimus immunosuppression in our patient cohort has resulted in earlier presentation of PTLD, with improved EBV monitoring likely to have contributed to prompt diagnosis.

O24

### HPV INFECTION AMONG VACCINATED AND UNVACCINATED ITALIAN YOUNG PEOPLE

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#### BACKGROUND-AIM

In Italy, HPV vaccination has been introduced as a preventive measure against HPV-related diseases, with adolescent as its primary target. HPV surveillance is necessary to monitor the impact and success of HPV immunization programs. We conducted a study aimed at evaluating the prevalence of HPV infection and HPV types distribution among young people using molecular test on urine samples.

#### METHODS

The study involved 384 (362 females; 22 males) young people aged 18-26 years located in 3 Italian Region (Lombardy, Liguria, Marche): 141 vaccinated (2-valent/4-valent HPV-vaccine) girls, 221 and 22 unvaccinated girls and boys, respectively. Informed consent and urine sample was obtained from each participant. HPV DNA was extracted from concentrated urine samples using the NucliSENS® easyMAG™ (bioMérieux bv, France) kit. HPV DNA was detected through PCR amplification of a 450 bp segment of ORF L1. All amplified fragments were subjected to viral type analysis using RFLP method.

#### RESULTS

HPV prevalence was 14.7% (53/362; IC95%:11.3-18.9%) and 22.7% (5/22; IC95%:8.7-45.8%) among girls and boys, respectively. Recruitment of the males is still ongoing. All HPV+ girls were sexually active. HPV infections were sustained by a broad spectrum of types: 42% of HPV+ girls showed multiple infection, 87% of which was sustained by at least one HR-clade HPV type. There was no difference in HPV prevalence in vaccinated and unvaccinated girls (14.2% vs 14.0%, respectively). 40% of unvaccinated HPV+ girls were infected by at least one HPV vaccine type (HPV-6, -16 or -18). No HPV vaccine types were identified in HPV+ vaccinated girls. 25% of HPV+ vaccinated girls and 40% of unvaccinated girls were infected with HPV types included in the 9-valent vaccine.

#### CONCLUSIONS

These data show a high HPV prevalence in vaccinated and unvaccinated Italian young people, confirming how young age and sexual activity represent risk factors for the acquisition of infection. In addition, the same HPV prevalence observed in vaccinated and unvaccinated girls indicates that a large number of HPV types are involved in HPV infections. The lack of infection by HPV vaccine types in vaccinated girls supports the effectiveness of vaccination. Therefore, the introduction of the 9-valent vaccine could help reduce a further significant rate of infection.

O25

### A PROSPECTIVE OBSERVATIONAL STUDY TO EVALUATE A CMV-SPECIFIC ELISPOT ASSAY IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (ALLO-HCT) RECIPIENTS: THE REACT STUDY

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#### BACKGROUND-AIM

CMV infection causes significant morbidity and mortality after allogeneic hematopoietic cell transplantation (allo-HCT) and is generally managed using a preemptive strategy with CMV viral load monitoring. CMV replication is primarily controlled by T-cell response which can be measured by quantifying IFN- $\gamma$  production. Therefore, we evaluated the role of a novel CMV-specific ELISPOT assay to predict CMV infection in allo-HCT recipients.

#### METHODS

This is a multi-center (13 sites), prospective, observational study of 244 adult CMV seropositive allo-HCT recipients. Significant CMV reactivation was defined as a positive blood PCR or antigenemia necessitating antiviral therapy according to each institutional protocols. T cell responses were serially monitored pre-, and every 2 weeks post-HCT and up to 26 weeks with an ELISPOT assay that uses CMV-specific antigens IE-1 and pp65 (T-SPOT.CMV, Oxford Diagnostics Laboratories®, Memphis, TN).

#### RESULTS

Majority of the patients are white (73%), male (56%), and the median age is 56 years (22 – 80). More patients (46%) had unrelated while 36% had matched HCT. Most of the donors (55%) were CMV sero-positive. CMV reactivation occurred in 61 patients (25%). The negative predictive value (NPV) of a pp65 spot count >100 was 92% and 85% for protection from first CMV reactivation and death, respectively. A Cox Proportional Hazards Model for time to CMV event showed that a maximum pp65 >100 was an independent predictor of protection (HR 0.139; CI 0.072 - 0.270; p-value <.0001) from CMV reactivation while systemic steroid use was an independent predictor (HR 4.186; CI 1.506 – 11.633; p-value <.006) of CMV reactivation.

#### CONCLUSIONS

The REACT study demonstrated that pp65 counts >100 was a significant predictor of protection against CMV reactivation. After adjusting for different risk factors, pp65 >100 was significantly associated with protection against CMV reactivation while the use of systemic steroids was significantly associated with CMV reactivation. This study suggests insights into the CMV immune response which may guide personalized decisions regarding CMV management.

O26

### AGE-DEPENDENT DYSREGULATION OF TYPE I IFN AND RETROVIRAL RESTRICTION FACTORS IN HIV-1 INFECTED PATIENTS ON LONG-TERM ANTIRETROVIRAL THERAPY.

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#### BACKGROUND-AIM

In order to understand how aging influences the IFN mediated immunity during HIV infection, the profile of expression of type I IFNs and the retroviral restriction factors, APOBEC3G, SAMDH1 and ISG15/UBP43, were compared among two age groups (30-50 and over 60-80 years) of treated HIV-1+ patients with stable virologic suppression. Furthermore, the functional integrity of IFN-signaling pathway was evaluated.

#### METHODS

PBMC from 150 long-term ART-treated HIV-1+ patients and from 65 gender and age matched healthy individuals were collected at the Sapienza University Hospital. Both the HIV-1+ patients and healthy subjects were divided into two groups: middle aged (age 30-50 years) and elderly (age 60-80 years). Levels of type I IFNs, ISG15, UBP43, APOBEC3G, SAMDH1-mRNAs were evaluated by Real Time RT-PCR assays. The same analysis was performed in lymphocytes CD4+ and monocytes CD14+ collected from two subgroups [middle aged (n=12), old (n=10)] of HIV-1 positive patients and in PBMC collected from two subgroups [middle age (n=5), old (n=5)] of HIV-1 positive patients after 24 hours of stimulation with IFN or Poly:IC.

#### RESULTS

In the healthy individuals, the levels of all the type I IFNs analysed were comparable between the two age groups, while the levels of all the restriction factors were increased in the old group compared to the middle aged. In the HIV-1+ patients the trend was different: a decreased expression of the IFN alpha/beta subtypes, ISG15 and UBP43 was observed in HIV-1+ elderly patients compared to the middle aged (p<0.01 for both genes). In contrast, both APOBEC3G and SAMDH1 levels tend to remain unchanged. Moreover, an increased expression of IFNs, ISG15 and UBP43 was recorded in lymphocytes compared to monocytes in the old group of HIV-1+ patients (p<0.05). No such differences were observed for APOBEC3G and SAMDH1. Lastly, we found that aging did not affect the ability to induce an IFN response after in vitro PBMC stimulation by Poly:IC and IFN.

#### CONCLUSIONS

Our results indicate that the expression of type I IFNs and the host restriction factors was differentially affected by ageing, being sustained in part by an IFN age-dysfunction in monocytes, and was not associated with a loss of functional integrity of IFN signaling pathway.

O27

### HUMAN PARECHOVIRUS 1, 3 AND 4 NEUTRALIZING ANTIBODIES IN DUTCH MOTHERS AND INFANTS AND THEIR ROLE IN PROTECTION AGAINST DISEASE

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#### BACKGROUND-AIM

Human parechoviruses (HPeVs) are common pathogens in young children, and in the Netherlands HPeV1, 3 and 4 are the most frequently detected genotypes. HPeV3 in particular has been associated with severe disease in young infants below the age of 3 months while the other genotypes more often infect older children and elicit mild symptoms.

#### METHODS

A prospective case-control study of Dutch mother-infant pairs was conducted to determine if maternal neutralizing antibodies (nAbs) against HPeV1, 3 and 4 protect young infants from severe disease related to HPeV infection. 38 HPeV-infected infants and their mothers were included as cases, and 65 HPeV-negative children and their mothers as controls.

#### RESULTS

In control infants we observed nAb seropositivity rates against HPeV1, 3 and 4 of 41.4%, 33.3% and 27.6% (median nAb titers 1:16, 1:12 and 1:8, respectively). In control mothers nAb seropositivity rates against HPeV1, 3 and 4 were 84.6%, 55.4% and 60.0% (median nAb titers 1:128, 1:32 and 1:45). The HPeV3 nAb seroprevalence was significantly lower in HPeV3-infected infants and their mothers (0.0% with p<0.05 and 10.0% with p<0.001, respectively). In contrast, no differences in nAb seroprevalence against HPeV1 or 4 could be detected between case and control infants or mothers.

#### CONCLUSIONS

Our results suggest that young Dutch infants are protected against severe disease related to HPeV1 and HPeV4 by maternal nAbs, but less so against HPeV3, explaining the distinct age distributions and disease severity profiles of children infected with these HPeV genotypes.

O28

# DEVELOPMENT AND EVALUATION OF A BEAD-BASED MULTIPLEX IMMUNOASSAY FOR IGG-SEROREACTIVITY AGAINST HUMAN POLYOMAVIRUSES

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## BACKGROUND-AIM

The family of polyomaviruses has increased substantially, and currently includes 13 human species (HPyV). For seroepidemiological purposes we extended our custom multiplex bead-based (Luminex) immunoassay to measure IgG antibodies directed against the major capsid protein VP1 of all currently known HPyV. In this study we evaluate this pan-polyomavirus immunoassay.

## METHODS

HPyV VP1 proteins were expressed as recombinant glutathione-s-transferase (GST)-fusion proteins and bound to glutathione-casein coupled Luminex fluorescent beads. VP1-directed antibodies were detected by phycoerythrin-labeled secondary antibodies. Sera from healthy blood donors (N=83) and immunocompromised kidney transplant recipients (N=65) were used to analyse seroresponses against 14 HPyV, and to determine reproducibility and specificity of the assay.

## RESULTS

A broad range of HPyV seroreactivities was observed. For most HPyV, high and frequent seroreactivity were observed, whereas for JCV and HPyV9 seroreactivity was generally low, and virtually absent for HPyV12 and NJPyV. The intertest reproducibility was high (Pearson's  $r_2 > 0.9$ ). Strong cross-reactivity was observed between HPyV10 and MWPyV, which belong to the same HPyV species (HPyV10). Weak cross-reactivity was observed between HPyV6 and HPyV7, and between JCPyV and BKPyV that are more distantly related. VP1 antigen-competition experiments largely confirmed these patterns.

## CONCLUSIONS

The custom HPyV-VP1 multiplex immunoassay measures HPyV-specific IgG seroreactivities with high reproducibility and limited cross-recognition. Therefore, the assay is a reliable tool to determine HPyV-specific seroprevalence in selected populations.

O29

# ENTEROVIRUS A71 OUTBREAK ASSOCIATED WITH SEVERE NEUROLOGIC DISEASES IN SPAIN, 2016

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## BACKGROUND-AIM

Enterovirus A71 (EV-A71) belongs to EV species A in genus Enterovirus, family Picornaviridae. EV-A71 was considered a low circulation neurologic virus, but in the late 1990s it emerged in eastern Asia causing large outbreaks of hand-foot-mouth disease (HFMD), associated with severe or fatal brainstem encephalitis, mainly in children. In Spain, EV-A71 had been rarely detected until 2015. However, in spring 2016 an EV-A71 outbreak associated with severe neurologic cases was reported in Catalonia, which spread to other regions. The aim of this study was to investigate the molecular epidemiology and clinical association of EV-A71 infections in Spain during 2016.

## METHODS

From January to December 2016, the National EV Laboratory received a total of 1099 EV-positive samples from patients admitted in different hospitals with suspicious of neurologic diseases -meningitis, encephalitis (E), brainstem-encephalitis (BE), acute flaccid paralysis (AFP) or encephalomyelitis (EM)-, fever, HFMD, respiratory illnesses and others. EV genotyping was performed by using 4 specific RT-PCRs for EV-A, B, C and D species, sequencing and phylogenetic analysis in the 3'-VP1 and 3Dpol regions.

## RESULTS

The most frequent serotype identified in 2016 was EV-A71 (231/826 typed EV, 28%). EV-A71 infections were in children (98%, mean age, 2.1 yr). The highest incidence was between May and July (84%). Clinically, severe neuropathologies (E, BE, AFP, EM) were more associated with EV-A71 than with other EV (68% vs 14%,  $p < 0.0001$ ). Infections were confirmed mainly in respiratory and/or stool samples (93%), but EV-A71 was also detected in CSF and serum (6%). 23% of the children with severe neurologic diseases required PICU and 65% received some treatment. Most of them (88%) had no significant sequelae. EV-A71 strains from 2016 were classified as subgenogroup C1 (detected for first time in Spain), highly related to recently described strain in Germany.

## CONCLUSIONS

Our results show that an emerging recombinant variant of EV-A71 C1 has been responsible for the large outbreak occurred in Spain during 2016 with many severe neurologic cases. These findings highlight the importance of EV surveillance in order to detect outbreaks early, as well as to identify new variants with increased pathogenicity.

## O30

### MOLECULAR EPIDEMIOLOGY OF ENTEROVIRUS INFECTION IN IRELAND 2015 – 2016

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## BACKGROUND-AIM

Enteroviruses (EV) are associated with a wide range of clinical syndromes, from rash, fever, and hand, foot, and mouth disease, to meningitis, acute flaccid paralysis (AFP), and acute flaccid myelitis (AFM). The National Virus Reference Laboratory (NVRL) is the national WHO Laboratory for Poliovirus/Enterovirus Surveillance for Ireland. In 2015, to complement traditional cell culture-based EV serotyping techniques, we implemented molecular genotyping directly from clinical samples to facilitate real-time detection and monitoring of circulating non-polio enteroviruses (NPEV).

## METHODS

A total of 11,713 clinical specimens was tested for EV RNA between January 1, 2015 and December 31, 2016. Specimens were tested using a real-time one-step reverse transcriptase (RT)-PCR assay for the detection of both EV and parechovirus (PeV) RNA. A proportion of EV positive clinical specimens was referred for genotyping using primers which target the VP1 region. Genetic analysis was performed by Sanger sequencing and genotypes were identified using the RIVM Enterovirus Genotyping Tool.

## RESULTS

EV RNA was detected in 11.7% of specimens (n=1375/11,713): more specifically, in 6.4% of CSF specimens (n=433/6792), 26.0% of faecal specimens (n=309/1187), 19.0% of throat swabs (n=185/976), and 12.9% of other specimen types. Although EV was found in all age groups, children aged < 1 year comprised the majority affected. Seasonal trends were observed as expected. Genotyping was performed on 21.6% of the positive specimens. Genetic characterisation revealed that the majority of sequences were members of EV species B (65.9%), followed by EV-A (32.2%), with EV-C (0.7%) and EV-D (1.1%) being the least common in circulation in Ireland during this period. The predominant genotypes included CV-A6, CV-B5 and CV-B1. Of note, EV-71 was identified in 8 specimens, and EV D68 was identified in 3 specimens.

## CONCLUSIONS

EV species A and B account for more than 98% of circulating EV in Ireland. As we move towards global polio eradication, and with increased numbers of EV-D68 and EV-A71 in circulation worldwide, both of which are linked to AFP and AFM, it is important to ensure prompt turn-around of genotyping results to provide real-time information to inform public health policy, and more importantly, to optimise patient care.

O31

### MOLECULAR EPIDEMIOLOGY OF ENTEROVIRUSES AMONG PATIENTS WITH ASEPTIC MENINGITIS IN SOUTH OF IRAN

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#### BACKGROUND-AIM

Viral infections are the major aetiological agent of aseptic meningitis; though, limited data exist on the prevalence and molecular epidemiology of viral pathogens responsible for the occurrence of aseptic meningitis in Iran. Therefore, this study aimed to investigate the presence of enteroviruses and their clinical significance, time distribution and age distribution among patients with aseptic meningitis who admitted to Shohadaie Khalij-Fars Hospital in Bushehr province.

#### METHODS

This study was funded by Bushehr University of Medical Sciences with grant number 4359. From June 2014 to August 2015, cerebrospinal fluid (CSF) specimen were obtained from 73 patients with aseptic meningitis (52.1% males and 47.9% females), ages ranging from 1 month to 88 years. Clinical data and informed consent at the time of CSF collection were obtained. Following extraction of nucleic acid, detection of enteroviruses was performed by RT-PCR, targeting the 5' untranslated region of the genome, and sequencing. This study was approved by the Ethical Committee of Bushehr University of Medical Sciences with reference number bpums. rec.1394.29.

#### RESULTS

The highest incidence of aseptic meningitis was reported in autumn and spring. The bacterial culture of all samples was negative. The most predominant clinical symptoms were fever, drowsiness, irritability, headache, rash and generalized weakness. Antibiotic therapy with empiric antibiotics was reported in 91.8% of patients. Enteroviruses were found in 34 (46.6%) of samples, from which echo 30 was the predominant subtype. The majority of enteroviruses infections was found among female patients (58.8%) and those below 5 years of age (52.9%).

#### CONCLUSIONS

The outcomes revealed that enteroviruses are significant causes of aseptic meningitis in South of Iran, while patients suspicion of meningitis are usually monitored by bacterial culture and biochemical testing of CSF samples. Therefore, the etiology remains unknown in most cases. Molecular detection of viral pathogens should be included as a common approach in the screening of patients with aseptic meningitis to prevent unnecessary treatment and to improve clinical management.

O32

### A SIX-YEAR EVALUATION OF VIRAL CENTRAL NERVOUS SYSTEM INFECTIONS

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#### BACKGROUND-AIM

Central nervous system (CNS) infections cause high mortality and morbidity and require rapid diagnosis. Application of nucleic acid tests to the diagnosis of CNS infections increased the identification of viral agents significantly. In this study, nucleic acid test results of cerebrospinal fluid (CSF) samples taken from clinically suspected CNS infection patients were evaluated for viral agents.

#### METHODS

2728 CSF samples taken between 2010 and 2016 tested for the presence of viral (HSV1/2, VZV, EBV, CMV, adenovirus and enteroviruses) nucleic acids by polymerase chain reaction (PCR) in Dokuz Eylul University Hospital. Tests were done according to the order of the clinicians and results were evaluated retrospectively. The number of samples tested for specific nucleic acids were as follows: HSV1/2 DNA in 824, adenovirus DNA in 699, enterovirus RNA in 672, VZV DNA in 399, EBV DNA in 89 and CMV DNA in 46 samples. Commercial tests were used for EBV, CMV (Artus QS-RGQ Kits, Qiagen, Germany) and enterovirus (GeneXpert Xpert EV, Cepheid, USA), while the other viruses (HSV, VZV, adenovirus) were tested by in-house real-time PCR assays.

#### RESULTS

CSF samples were taken from 921 patients; 54.7% (n: 504) of whom were male and 45.3% (n: 417) were female. Sixty one of the 2728 CSF samples (2.2%) were positive for one or more tested viral agent which was equal to 6.5% (61/921) of the patients. The most frequently detected pathogens were enterovirus (41/61, 67%) and HSV type 1 (8/61, 13%). The number of patients that were positive for adenovirus, VZV, EBV and CMV were 4, 4, 3 and 1 respectively. In one patient, both enterovirus RNA and EBV DNA were positive. The number of positive samples was peaked in June (9.8%), July (16.4%) and August (14.8%). When enterovirus-positive patients were evaluated, 25% were younger than one year of age. Enterovirus positivity was detected mainly in summer (48.8%) and autumn (24.4%) months. HSV type 1 and VZV are detected mainly in patients above 65 years of age.

#### CONCLUSIONS

In the six-year period, among the 921 patients, 6.5% of were positive for viral nucleic acid in CSF samples. The low rate of positive samples could be related to selected pathogen testing according to clinicians order. Syndromic screening could increase the pathogen detection.

O33

### FAST AND RELIABLE NGS-BASED METHOD FOR INVESTIGATION OF NOROVIRUS OUTBREAKS WITH SINGLE OR MULTIPLE GENOTYPES

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#### BACKGROUND-AIM

Norovirus is the leading cause of gastroenteritis. Fast and reliable outbreak investigations are crucial in order to stop the chain of infection. Investigation of outbreaks caused by mixed genotypes is hampered by Sanger sequencing due to limitations of detecting mixed nucleotides. We developed a new NGS-based method and compared the results to Sanger sequences from artificial mixtures and samples from a hitherto unsolved outbreak

#### METHODS

Samples, with known genotypes, of similar Ct were used to mix samples. Three mixtures were used I) GI-GI, II) GII-GII and III) GI-GII. In addition, samples from an unsolved outbreak were analysed. A fragment of approx. 1100 nt spanning the ORF1-ORF2 junction was amplified using forward ORF1 primer JV12 (GI and GII) and reverse ORF2 primer G1SKR (GI, mixture I) or G2SKR (GII, mixture II). For mixture III, PCR reactions were performed using an all three primer mix. Two sequencing approaches were used: 1) Sanger and 2) NGS using a MiSeq. NGS data was analysed by CLC Genomics Workbench. Genotyping was performed on NoroNet

#### RESULTS

Single primer pair PCR's showed stronger bands than three primer reactions. Sanger sequencing detected only the dominating genotype and with mixed chromatograms. One sample gave a combination of the two genotypes indicating a recombinant. NGS data assembly to a reference collection was more reliable than de novo assembly. In all samples correct consensus fragments were detected. In de novo assembly the contig was sometimes of incorrect size. Even though PCR products were weaker in reactions containing all three primers (Mixture III) analysis of the NGS data showed very good recovery of the expected genotypes in the expected ratio. Using this approach on outbreak samples with mixed genogroups and genotypes, it was possible to connect all samples as part of a common outbreak via overlapping genotypes found in the different samples

#### CONCLUSIONS

The presented NGS approach showed to be fast and reliable for genotyping norovirus, even in mixed samples. This may prove very beneficial in solving norovirus outbreaks where Sanger sequencing gives no applicable results. Using a single three primer PCR reaction spanning the POL-CAP intergenic region makes this method less time and resource consuming than the traditional method

O34

### EMERGING G3 ROTAVIRUSES IN EASTERN INDIA REVEALED INTERGENOGROUP RECOMBINATION AND CHANGES IN ANTIGENIC EPITOPES DURING 2014-2016.

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#### BACKGROUND-AIM

Advent of new strains and shift in predominantly circulating genotypes are characteristics of human group-A rotaviruses (GARV), an etiological agent of infantile gastroenteritis. During diarrheal disease surveillance at Kolkata (2014-2016), a shift in circulating GARV strains from G1P[8] to G3P[8] was seen in 2015. In 2016, G3 became the predominant genotype, though in past decade G3 strains were not observed in Eastern India. To gain insight into the evolution of G3 viruses, we conducted whole genome analyses of representative G3P[8] and G3P[4] strains. This study is important as efficacy of rotavirus vaccines depends on circulating heterogeneous genotype constellations.

#### METHODS

RNA was extracted from 3048 stool samples by Qiagen viral RNA isolation kit. Complementary DNA was synthesized through reverse transcription and all eleven gene segments were amplified by PCR using gene specific primers, followed by sequencing using Sanger's method. Genotyping were done through BLAST analysis. Phylogenetic tree was constructed using MEGA6 software.

#### RESULTS

In 2014-2016, 38.7% were GARV positive among 3048 stool samples from children with acute gastroenteritis. In 2014-2015, among 759 GARV positives, G1 was the predominant strain (65.3%); while in 2015-2016 of 746 GARV positives, G3 became the preponderant strain (44.6%). Whole genome analyses revealed intergenogroup reassortment in G3P[4] strains (between Wa and DS-1-like genogroup) bearing VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 genetic backbone of I1-R1-C1-M1-A1-N1-T2-E1-H1, and pure Wa-like G3P[8] strains with I1-R1-C1-M1-A1-N1-T1-E1-H1 backbone. Phylogenetic analysis revealed genetically polymorphic Kolkata G3 strains being divided into two sub-clusters marked by the presence of antigenic differences. Sub-cluster I had the wild-type threonine while sub-cluster II had a fitness advantage by having an isoleucine mutation. The extra N-linked glycosylation site at amino acid 283 of VP7 protein demonstrates that the major neutralizing epitope of RotaTeq vaccine differs from the currently circulating G3 strains.

#### CONCLUSIONS

The results suggest continuous monitoring of circulating GARV strains with a goal to explicate the significance of reassortants and mutants contributing to the viral fitness and assessing viral efficacy.

O35

### REPLICATION IN HUMAN INTESTINAL ENTEROIDS TO ASSESS HUMAN NOROVIRUS INACTIVATION

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#### BACKGROUND-AIM

Human noroviruses are the most common cause of epidemic gastroenteritis worldwide and a leading cause of foodborne illness in the US. Due to the lack of a robust cell culture system, evaluation of inactivation methods and products have relied on the use of cultivable surrogate viruses, with variable results depending on treatment type and which surrogate virus is used. Recently, non-transformed human intestinal enteroids (HIEs) produced from three-dimensional (3D) in vitro cultures derived from proliferating crypts isolated from small intestinal tissue of humans have been shown to support replication of human norovirus. The aim of this study was to test the effect of chlorine and alcohols on infectious human norovirus.

#### METHODS

Thirty-nine RT-qPCR positive stool filtrates (Ct range: 14-35; GI n=3; GII.4 n=33; GV n=3) were tested on jejunal HIE monolayers. Three GII.4 strains (Den Haag, New Orleans and Sydney) that replicated were treated with increasing concentrations (0, 5, 50, 100, 200, 400, 600, 800, 1000, and 5000 ppm) of chlorine for 1 min at room temperature. Inactivation efficacy of 70% ethanol or isopropanol was also tested. Norovirus infectivity was determined after 72 h post infection by realtime RT-PCR and copy numbers were determined using RNA-transcripts.

#### RESULTS

Of 39 stool samples that were tested on the HIE monolayers, seven samples (all GII.4) showed a 102-103 fold increase of genome equivalents per well. At least two samples could be passaged successfully into passage 2. Complete inactivation of three GII.4 viruses was observed at concentrations as low as 50 ppm of chlorine. Although both ethanol and isopropanol reduced virus infectivity, complete virus inactivation was not achieved regardless of type of alcohol, concentration or exposure time.

#### CONCLUSIONS

Our data confirm a previous report of successful replication of several GII.4 noroviruses using monolayers of jejunal HIEs and further demonstrate that chlorine concentrations as low as 50 ppm are capable to inactivate human norovirus. This new HIE-based biologically relevant replication system permits human host-pathogen studies of human norovirus, and allows the assessment of methods to prevent and treat human norovirus infections.

O36

### HUMAN ASTROVIRUSES: INSIGHTS OF A SIX-YEAR MOLECULAR EPIDEMIOLOGICAL ANALYSIS IN GERMANY

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#### BACKGROUND-AIM

Human astroviruses (HAstV) are important enteric pathogens which are associated with acute gastroenteritis predominantly in children, but also adults and elderly can be affected. Astroviruses are divided into two genera, the Mamastrovirus (MAstV) infecting mammals and the Avastrovirus (AAstV), infecting birds. In humans, four different MAstV species are known (MAstV-1, MAstV-6, MAstV-8 and MAstV-9). In order to analyze the molecular epidemiology of HAstV in Germany, a retrospective long-term study was performed using genetic characterization of astroviruses in patients with acute gastroenteritis (AGE) from 2010 to 2015.

#### METHODS

A total of 2877 stool samples of gastroenteritis outbreaks and sporadic cases in Germany were initially tested for norovirus (NV) and/or rotaviruses (RV) between January 2010 and December 2015. Retrospectively, these samples were analyzed for HAstV-genomes using RT-PCR techniques to determine the rate of positive samples in the study population. Phylogenetic analysis was performed to characterize the diversity of genotypes and their circulation pattern.

#### RESULTS

Overall, 143 of 2877 samples were HAstV positive (5%). Notably, the highest rate of HAstV infection was detected in 2013 (47/387, 12.1%). HAstV infection was most frequently detectable in samples from children 3-4 years (15/100, 15%). In contrast, in adults and elderly (20 years to >80 years) from seven different age groups HAstV genomes were detectable from 1% (age group 70-79) to 3.6% (age group 50-59). Co-infections with other gastroenteritis viruses were detected in 102/143 (71.3%) of the samples with mainly NV or RV. Genotyping revealed that at least eight genotypes from all four human MAstV species were circulating in the study population. HAstV-1 was the most prevalent genotype affecting patients of different age groups in all seasons. Novel HAstV (MAstV-6, MAstV-8 and MAstV-9) were detected in 2011 and from 2013 to 2015.

#### CONCLUSIONS

Our findings give new insights into the molecular epidemiology of human astroviruses in German patients with AGE.

O37

**ASSOCIATION OF RIFT VALLEY FEVER VIRUS INFECTION WITH MISCARRIAGE IN HUMANS**M. Baudin <sup>2</sup>, A. Jumaa <sup>3</sup>, H. Jomma <sup>4</sup>, M. Karsany <sup>5</sup>, G. Bucht <sup>6</sup>, J. Näslund <sup>6</sup>, C. Ahlm <sup>1</sup>, M. Evander <sup>2</sup>, N. Mohamed <sup>2</sup><sup>1</sup>Department of Clinical Microbiology, Infectious Diseases, Umeå University, Umeå, Sweden<sup>2</sup>Department of Clinical Microbiology, Virology, Umeå University, Umeå, Sweden<sup>3</sup>Department of Obstetrics and Gynaecology, Red Sea University, Port Sudan, Sudan<sup>4</sup>Department of Parasitology and Medical Entomology, Port Sudan Ahlia College, Port Sudan, Sudan<sup>5</sup>Faculty of Medical Laboratory Sciences, Karary University, Khartoum, Sudan<sup>6</sup>Swedish Defence Research Agency, CBRN Defence and Security, Umeå, Sweden**BACKGROUND-AIM**

Rift Valley fever virus is an emerging mosquito-borne virus that causes infections in animals and humans in Africa and the Arabian Peninsula. Outbreaks of Rift Valley fever lead to mass abortions in livestock, but such abortions have not been identified in humans. Our aim was to investigate the cause of miscarriages in febrile pregnant women in an area endemic for Rift Valley fever.

**METHODS**

Pregnant women with fever of unknown origin who attended the governmental hospital of Port Sudan, Sudan, between June 30, 2011, and Nov 17, 2012, were sampled at admission and included in this cross-sectional study. Medical records were retrieved and haematological tests were done on patient samples. Presence of viral RNA as well as antibodies against a variety of viruses were analysed. Any association of viral infections, symptoms, and laboratory parameters to pregnancy outcome was investigated using Pearson's  $\chi^2$  test.

**RESULTS**

Of 130 pregnant women with febrile disease, 28 were infected with Rift Valley fever virus and 31 with chikungunya virus, with typical clinical and laboratory findings for the infection in question. 15 (54%) of 28 women with an acute Rift Valley fever virus infection had miscarriages compared with 12 (12%) of 102 women negative for Rift Valley fever virus ( $p<0.0001$ ). In a multiple logistic regression analysis, adjusting for age, haemorrhagic disease, and chikungunya virus infection, an acute Rift Valley fever virus infection was an independent predictor of having a miscarriage (odds ratio 7.4, 95% CI 2.7–20.1;  $p<0.0001$ ).

**CONCLUSIONS**

This study is the first to show an association between infection with Rift Valley fever virus and miscarriage in pregnant women. Further studies are warranted to investigate the possible mechanisms. Our findings have implications for implementation of preventive measures, and evidence-based information to the public in endemic countries should be strongly recommended during Rift Valley fever outbreaks.

O38

**WHAT IS UNDERLYING HYPERECHOGENIC BOWEL IN CONGENITALLY CYTOMEGALOVIRUS INFECTED FETUSES?**L. Gabrielli <sup>2</sup>, M.P. Bonasoni <sup>5</sup>, D. Santini <sup>6</sup>, G. Simonazzi <sup>4</sup>, M.G. Capretti <sup>3</sup>, I. Tamagnini <sup>5</sup>, A. Chiereghin <sup>2</sup>, G. Piccirilli <sup>2</sup>, C. Pavia <sup>2</sup>, G. Turello <sup>2</sup>, D. Squarzone <sup>2</sup>, T. Lazzarotto <sup>1</sup><sup>1</sup>Operative Unit of Clinical Microbiology, Department of Specialized, Experimental, and Diagnostic Medicine, University of Bologna, St. Orsola-Malpighi University Hospital, Bologna, Italy<sup>2</sup>Operative Unit of Clinical Microbiology, St. Orsola-Malpighi University Hospital, Bologna, Italy<sup>3</sup>Operative Unit of Neonatology, St. Orsola-Malpighi University Hospital, Bologna, Italy<sup>4</sup>Operative Unit of Obstetrics and Prenatal Medicine, St. Orsola-Malpighi University Hospital, Bologna, Italy<sup>5</sup>Operative Unit of Pathology, St. Maria Nuova Hospital, Reggio Emilia, Italy<sup>6</sup>Operative Unit of Pathology, St. Orsola-Malpighi University Hospital, Bologna, Italy**BACKGROUND-AIM**

Hyperechogenic bowel is diagnosed by ultrasound in 1.4% of pregnancies during the second trimester, and has been associated with cystic fibrosis, chromosomal abnormalities, and in utero infections such as cytomegalovirus (CMV).

The aim of our study was to find a possible pathophysiology underlying hyperechogenic bowel in congenital CMV infected fetuses.

**METHODS**

We examined small and large intestines as well as pancreas in 8 fetuses at 22 weeks of gestation with congenital CMV infection. Fetal diagnosis of CMV infection was based on CMV positivity in amniotic fluid by culture and Real Time PCR. Ultrasound findings showed four fetuses with hyperechogenic bowel and four without ultrasound anomalies. Serial sections of duodenum, jejunum, ileum, large bowel and pancreas were submitted for histological examination. Immunohistochemistry for CMV and lymphocytic infiltrate were also performed.

**RESULTS**

In the 4 fetuses with hyperechogenic bowel, macroscopic autopsy showed dilatation of the distal intestine, especially ileum and large bowel. In addition, meconium appeared thickened and distally localized. Microscopic examination showed intestinal ganglioneuritis with CMV positive cells in the intestinal ganglia surrounded by a T lymphocytic infiltrate. CMV positive ganglion cells were observed only in the Auerbach's myenteric plexus throughout all various intestinal regions. Moreover, meconium granules within the enterocyte cytoplasm facing the intestinal lumen were observed. These may represent indirect signs of reduced intestinal peristalsis. Pancreas was grossly normal, however at histology, epithelial cells were CMV positive, mostly surrounded by T lymphocytes. In the 4 fetuses with no hyperechogenic bowel, macroscopically, the intestine was not dilated and intestinal sections showed either no CMV cells or inflammatory infiltrate. However, pancreatic histology showed CMV positive cells and lymphocytic infiltrate similarly observed in fetuses with hyperechogenic bowel.

**CONCLUSIONS**

CMV may be responsible for the ileus which is probably paralytic and due to intestinal plexus involvement. Hyperechogenic bowel can probably be explained primarily as reduced intestinal motility due to CMV ganglioneuritis in the Auerbach's myenteric plexus instead of impairment in pancreatic enzyme secretions.

O39

### CONSEQUENCES OF MMR VACCINATION GAPS IN YOUNG ADULTS: MEASLES INFECTIONS IN PREGNANCY AND IN THE NEWBORN

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#### BACKGROUND-AIM

Despite great efforts made towards controlling measles in Austria by increasing vaccination coverage, the WHO target of elimination has not been achieved yet. In the last years measles outbreaks occurred repeatedly by introduction of different measles virus (MV) strains from other countries. The majority of cases emerged in the age groups of adolescents and young adults, indicating immunity gaps in this population. This shift towards the reproductive age leads to an increased risk of acquiring measles during pregnancy, which can have deleterious effects on mother and child.

#### METHODS

The Center for Virology, MUW, serves as the National MMR Reference Center (NRC), verifying the majority of reported cases. For this purpose, a broad spectrum of diagnostic tests has been established, including IgM- and IgG-ELISA, IgG-avidity assay, neutralization test, real time PCR, sequencing, and genotyping procedures.

#### RESULTS

Between 2013 to 2016, 13 measles infections in infants (<1a) were verified at the NRC including 3 infections of newborns. A detailed diagnostic follow-up was performed in mothers and infants. This included the detection of the antibody response and the viral load measurement from serum, oral-fluid, urine, and breastmilk samples. The 1st case was a 30 year old pregnant woman who got measles at gestation week 37 and was hospitalized. After 6 days she developed severe atypical pneumonia and gave birth while still being infectious. An intrauterine infection of the newborn was diagnosed by MV detection in a serum sample taken immediately after birth. In the 2nd case the mother developed measles 4 days post partum (pp). At this point MV was already detectable in serum and oral fluid of the newborn indicating a pre-or perinatal MV infection. The 3rd mother was infected probably on the day of delivery and developed rash on day 9 pp. Her infant came down with measles on its 18th day of life complicated by pneumonia.

#### CONCLUSIONS

Closing the MMR vaccination gaps is of major importance, especially in young adults, to reduce infections in pregnancy associated with a higher risk of hospitalizations, prematurity, and complications like pneumonia, low birth weight, and the subsequent development of SSPE in the worst case.

O40

### DEVELOPMENT OF PAN-PARECHOVIRUS ANTIBODIES FOR POINT-OF-CARE

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#### BACKGROUND-AIM

Human parechoviruses (HPeV) are common picornaviral pathogens in children with high seroprevalence before the age of five. Currently, nineteen (19) parechovirus types are known. Parechoviruses have traditionally been linked to gastroenteritis and respiratory infections, and more recently to severe CNS disease especially in infants. Currently, there are no other means for the detection of HPeVs but RT-qPCR. The aim of this study was to develop diagnostic antibodies for the detection of human parechoviruses in a novel point-of-care assay setup.

#### METHODS

We used both purified human parechovirus 1 (HPeV-1) and recombinant VP0 protein produced in *E. coli* (HPeV-1-VP0) as targets in antibody generation (traditional monoclonal and scFv antibody library production systems).

#### RESULTS

Sequence alignment suggested VP0 as target for such site. HPeV-1 (virus) and HPeV-1-VP0 (protein) were then used as mouse immunogens to obtain hybridoma cell lines; One thousand hybridoma clones were screened and two pan-parechovirus clones were identified. The same antigens were used in biopanning approach (against human phage display scFv antibody library) and three pan-parechovirus binders possessing different CDR3 sequence were identified. The specificities of the antibodies were tested on ELISA and IFA. The performance of pan-HPeV antibodies at point-of-care setting is currently being evaluated (mariPOC platform from ArcDia International Ltd.).

#### CONCLUSIONS

In all, pan-parechovirus antibodies have been developed both using traditional monoclonal and biopanning approaches.

O41

### ADDED VALUE OF ULTRA-DEEP SEQUENCING APPROACH FOR DETECTION OF GENOTYPIC ANTIVIRAL RESISTANCE OF HERPES SIMPLEX VIRUS

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#### BACKGROUND-AIM

Acyclovir (ACV) constitutes the first-line therapy for herpes simplex virus (HSV) infections. Targeting viral DNA polymerase (Pol, UL30 gene), ACV activity depends on the phosphorylation by the virus-encoded thymidine kinase (TK, UL23 gene). TK alterations, either nucleotide insertions/deletions leading to translational frameshift or nucleotide substitutions, account for 95% of HSV resistance to ACV, remaining cases corresponding to nucleotide substitutions in Pol. Classically, TK and Pol gene Sanger sequencing is the gold standard for detection of drug resistance mutations (DRMs). However, this approach cannot detect minor DRMs in the viral population. As a complementary method, ultra-deep sequencing (UDS) has an improved ability to detect minor variants and mixed populations. The aim of this work was to develop UDS for the detection of HSV DRMs and to evaluate the subpopulation diversity in clinical samples.

#### METHODS

Viral DNA was extracted from 59 HSV-positive clinical samples recovered from patients experiencing treatment failure. UL23 and UL30 genes were sequenced in parallel by Sanger and UDS methods using shotgun strategy on the MiSeq® platform (Illumina). With this approach, reproducible detection of mutations present in at least 10% of the viral population was achieved.

#### RESULTS

UDS and Sanger results were fully concordant. Few unknown mutations potentially associated with resistance were identified as minor variants by UDS. All single DRM identified in TK or Pol with Sanger method was detected as high-abundant mutations with UDS ( $\geq 91.5\%$ ). Along the same lines, TK frameshifts were systematically detected at lower abundance ranging from 48.7% to 92.9% with UDS. For some samples, Sanger method revealed distinct DRMs in either TK or Pol, for which low-abundance frequencies were showed by UDS, supporting the idea of a heterogeneous viral population in those cases.

#### CONCLUSIONS

This work revealed a complex distribution of HSV DRMs. UDS can detect low-frequency mutations and provides extensive information on viral population composition. Our data showed a significant difference of relative abundance according to the type of DRMs. Our data legitimate the implementation of UDS for HSV resistance monitoring.

O42

### EPSTEIN BARR VIRAL MICRORNAS PROFILING OF PROGNOSTIC AND DIAGNOSTIC VALUE IN A PTLD PATIENT COHORT

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#### BACKGROUND-AIM

Post-transplant lymphoproliferative disorder (PTLD) is a common and serious complication (2%–10% of post-transplant patients) of solid organ and hematopoietic stem cell transplantation, and in the 80% of the cases have been associated with Epstein-Barr virus (EBV) infection. Lately, many studies have focused their attention on viral miRNAs and their functions on prolonging longevity of infected cells, evading the immune response, and regulating host or viral genes to limit the lytic cycle. The virus encode for two clusters of viral miRNAs are BHRF (latency phase III) and BART (still unknown function). Here, we investigate the EBV miRNAs signature in a PTLD patient cohort in order to shed a light in the prognostic and diagnostic value that viral miRNAs can play in the PTLD pathogenesis.

#### METHODS

We profiled the viral miRNome (44 microRNAs) using microfluidic cards (qRT-PCR), in two different cohort of patients that underwent lung transplantation, PTLD and control, at different time points (-3, -1 and at the moment of the diagnosis).

#### RESULTS

We profiled the whole EBV miRNome (44 microRNAs) in two different cohort, PTLD and control, at different time points (-3, -1 and at the moment of the diagnosis). We have selected 4 PTLD patients with similar transplant characteristics (lung transplant) and as control group, 4 lung transplant patients that have never developed PTLD. We found that one microRNA in particular, ebv-miR-BART7, is significantly differentially expressed in the PTLD population comparing to the controls. It is notable that we found this miRNA differentially expressed even 1 and 3 months before the diagnosis.

#### CONCLUSIONS

PTLD is a well-recognized complication of both solid organ and allogeneic hematopoietic stem cell transplantation and is associated with EBV infection of B cells. Here, we investigate the EBV miRNAs signature in a PTLD patient cohort and we found that a viral microRNA, ebv-miR-BART7, is significantly differentially expressed in the PTLD population comparing to the controls even 1 months before the diagnosis. This finding is particularly important because suggests a potential role for ebv-miR-BART7 as prognostic factor for PTLD and could help in a more accurate risk stratification for PTLD.

# POSTERS

001

## **VIROLOGICAL SURVEILLANCE OF RESPIRATORY ENTEROVIRUS FROM ATTENDED PATIENTS AT A TERTIARY HOSPITAL IN CATALONIA (SPAIN) DURING THE 2014-2017 SEASONS**

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### **BACKGROUND-AIM**

Enterovirus (EV) infections in humans are usually asymptomatic, but symptomatic infections sometimes can evolve to severe complications. There are more than 100 different types of human EV distributed within 4 species (A, B, C and D). Outbreaks of EV-A71 and EV-D68 have been recently reported worldwide, which might be related to severe clinical outcomes. The aim of the present study was to study the EV genetic diversity from patients attended at Vall d'Hebron University Hospital from 2014 to 2017 seasons.

### **METHODS**

From October 2014 to May 2017 including the 2015 and 2016 inter-seasonal periods, respiratory tract specimens were collected from patients attended at our hospital with suspicion of respiratory tract infection for respiratory viruses detection. EV detection was carried out by specific real-time multiplex RT-PCR assays (Anyplex RV16 II Detection and Allplex Respiratory Panel Kits, Seegene, Seoul, South Korea). Partial viral protein VP1 was additionally sequenced for further genetic characterisation by phylogenetic analyses.

### **RESULTS**

A total of 19,538 specimens from 12,439 cases were received for respiratory virus confirmation, of which 494 (4%) cases were EV laboratory-confirmed. Phylogenetic analyses of the partial VP1 sequences showed that 92 (32%) were EV-A, 93 (32%) EV-B, 5 (2%) EV-C, 35 (12%) EV-D, and in addition, 42 (15%) RV-A, 2 (<1%) RV-B and 14 (5%) RV-C. Moreover, a high diversity of types within each EV specie was found. The most predominant types by specie were EV-A71 (47; 51%), CV-A6 (12; 13%) and CV-A2 (12; 13%), for EV-A; CV-B3 (12; 13%), E-30 (11; 12%), E-5 (10; 11%) and CV-A9 (9; 10%) for EV-B; EV-C109 (5; 100%) for EV-C; and EV-D68 (34; 100%) for EV-D.

### **CONCLUSIONS**

During the study period, EV-A and EV-B were the most frequently detected species, jointly with EV-A71 and EV-D68 within each EV specie. The majority of EV-A71 were detected during the 2016 outbreak of rhombencephalitis associated with this type in Catalonia, whereas, EV-D68 were only detected during 2016 and none during 2015 after the North-American outbreak in 2014. Due to the severity of the clinical outcomes which these EV might be related to, it would be interesting to monitor its circulation.

002

## **HUMAN PARECHOVIRUS: A COMMON THREAT TO CHILDHOOD HEALTH (IF YOU DON'T LOOK YOU WON'T FIND IT)**

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### **BACKGROUND-AIM**

Infections with human parechoviruses (HPeVs) are highly prevalent, particularly in neonates, where they may cause substantial morbidity and mortality. The clinical presentation of HPeV infection in neonates varies from mild disease to severe illness and HPeV is one of the most common single causes of aseptic meningitis/meningoencephalitis in young infants worldwide. We reviewed the global HPeV-associated disease burden, biology of HPeV infection, state-of-the-art diagnostics and molecular epidemiology.

### **METHODS**

Literature review of existing data on HPeV disease and risk-factors (including seroprevalence and RNA-detection from surveillance data, and registry data and/or case-based studies on hospitalization and mortality burden), distribution of HPeV subtypes and diagnostic as well as subtype characterization methods.

### **RESULTS**

Published data on HPeV incidence in large populations is sparse and the majority of these are either based on enterovirus (EV) surveillance data or case-based. HPeV is transmitted both respiratory and fecal-orally. Risk factors of HPeV infections are related to young age and sibling status. State-of-the-art diagnostics involves detection of virus RNA. Some HPeV types are associated with a more severe course of infection, for example over 10% of HPeV3 infected infants require intensive care admission, and hence typing plays an important role in outbreak investigation and surveillance.

### **CONCLUSIONS**

Laboratory diagnosis of these viral infections is important not only for differential diagnostic purposes and determining a patient's prognosis but also for guidance of clinical management. Taking the global incidence of reported laboratory-confirmed HPeV cases into consideration, mortality and sequelae are overall uncommon and usually accompanying initially severe or neurologically complicated acute illnesses. However, underreporting due to lack of testing and/or appropriate diagnostic methodology likely means gross underestimation of the true incidence of HPeV disease. Currently, neither effective treatment nor vaccines are available in the control of these common viruses with the potential to cause severe harm in neonates and young children worldwide.

003

# **DIFFERENT INFLUENZA A H3N2 SUBCLADES CIRCULATING IN THE 2016/2017 SEASON AND LOW VACCINE EFFECTIVENESS IN PREVENTING HOSPITALISATION FOR LABORATORY-CONFIRMED INFLUENZA. RESULTS OF VALENCIA HOSPITAL NETWORK FOR THE STUDY OF INFLUENZA AND RESPIRATORY**

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## **BACKGROUND-AIM**

We summarize the results of influenza vaccine effectiveness (IVE) and the impact of previous influenza vaccination among subjects 60+ years old in the Valencia Region, Southeast of Spain.

## **METHODS**

Test-negative design taking laboratory-confirmed influenza as outcome and vaccination status as exposure. Consecutive emergency admissions for influenza-like illness (ILI), were screened in 4 tertiary-care hospitals serving to 22% of the 4,860,874 inhabitants. Nasopharyngeal and pharyngeal swabs were collected and tested by RT-PCR. Influenza-positive isolates with Ct≤26 were selected for hemagglutinin (HA) sequencing.

## **RESULTS**

A total of 196 admissions (17.9%) were positive for influenza A: 175 (89.3%) were influenza A(H3N2). The genetic characterization of 63 sequenced influenza A(H3N2) samples showed few clade 3C.3a (A/Switzerland/9715293/2013-like) viruses (n=3), with the majority (n=60) corresponding to the same clade (3C.2a) as the A/Hong\_Kong/4801/2014 2016-17 vaccine strain. However, 82% (n=49) of clade 3C.2a viruses belong to subclade 3C.2a1 (N171K), and four subgroups were characterized, each with a particular subset of mutations: 1) A/Bolzano/7/2006-like (n=14); 2) a local cluster (n=9); 3) A/Norway/3806/2016-like (n=8); and 4) A/Norway/4395/2016-like (n=18). Finally, from the remainder eleven clade 3C.2a isolates, four were related to A/Antsirabe/2047/2016, and the rest belonged to a new cluster characterized by the N121K+N122D+S144K mutations. Viral isolates from vaccinated individuals did not aggregate to any particular genetic group. IVE analysis was restricted to 60 years old or older, and adjusted IVE was 19% (95% CI: -15 to 43%). For patients vaccinated in the current season but not in the two previous seasons, effectiveness is 49% (-20 to 78%) and for patients vaccinated in the current and any of two previous seasons, effectiveness is 29% (-3 to 52%). For those patients not vaccinated in the current season but vaccinated in any of the two previous seasons, effectiveness is 53% (8 to 76%).

## **CONCLUSIONS**

These data show a low vaccine effectiveness for the 2016/17 influenza season, characterised by the circulation of A(H3N2) viruses belonging to a new emerging genetic subclade 3C.2a1, A/Bolzano/7/2016-like with heterogeneous genetic subgroups.

004

# ENTEROVIRUS D68 (EVD68) IN CHILDREN WITH ACUTE RESPIRATORY TRACT INFECTION (ARI) ADMITTED TO A UNIVERSITY AND RESEARCH HOSPITAL IN MILAN (ITALY), FROM 2015 TO 2016

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## BACKGROUND-AIM

Enteroviruses (EVs) are recognized to cause mild-to-severe acute respiratory tract infection (ARI), a common illness among children. EVD68 has been identified as a worldwide emerging pathogen associated with severe respiratory symptoms since 2009.

We report EVs and EVD68 detection in respiratory samples collected from children with ARI admitted to a University and research hospital in Milan (Italy), between January 2015 and December 2016.

## METHODS

Respiratory samples (nasopharyngeal swabs, aspirates or broncho-alveolar lavages) were collected from 1638 children <15 years (median age: 10.7 months; IQR: 36 months) admitted to hospital with ARI; 50.4% were collected in 2015 (825/1638) and 49.6% (813/1638) in 2016. Overall, 45.5% (746/1638) of cases occurred during winter months; 78.5% (1286/1638) cases required admission to intensive care unit and were classified as severe ARI (SARI).

After nucleic acids extraction (EZ1@DSP Kit, Qiagen), samples were tested for EV-RNA presence by multiplex real-time PCR (Anyplex™ II, RV16-Detection, Seegene); EV-positive specimens were analysed by real-time RT-PCR assay specific for EVD68.

## RESULTS

8.8% (144/1638) of samples were EV-positive. The median age of EV-positive children was 20.9 months (IQR: 37.2 months). 57.6% (83/144) of these were detected in 2016: the risk of EV infection was higher in 2016 than in 2015 (10.2% vs 7.4%; OR: 1.4; 95%CI: 1.1-2). Most (119/144: 82.6%) EV-positive samples were from SARI cases; 41.7% (60/144) occurred during summer months. Two-thirds (95/144: 66%) of EV-positive samples were tested for EVD68; among these, 19 (28.8%) were identified as EVD68. Nearly 90% (16/19) were from SARI cases. All but one (18/19: 94.7%) were identified in 2016, mostly (14/18: 77.8%) between July and October.

## CONCLUSIONS

In this study, an EV infection was identified in nearly 9% of children admitted to hospital with respiratory illness. EV infections mostly occurred in children <3 years and brought to severe infection in >80% cases; the risk of EV infection was higher in 2016. EVD68 was detected in 2016 - mainly during the summer - causing severe respiratory infections. The set-up of a surveillance system to monitor the spread and clinical impact of EVs and particularly EVD68 is strongly recommended.

005

# RSV INFECTIONS DURING 2014 -2016 EPIDEMICS: CLINICAL AND SEQUENCING CHARACTERISTICS IN UNIVERSITY HOSPITAL OF LYON, FRANCE.

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## BACKGROUND-AIM

Considered as one of the main cause of death in children before the age of five, the Respiratory Syncytial Virus (RSV) has been described as the agent responsible for almost 80% of the newborn bronchiolitis. Epidemiological season 2016-17 has been described as original with co-circulation of two main respiratory pathogens, Influenza and RSV, justifying the need for this type of study.

This study aimed to characterize retrospectively the clinical and phylogenic presentation of RSV during the three last winter seasons at the hospitals of Lyon, France.

## METHODS

All RSV-positive respiratory samples received at the virology laboratory of the Hospices Civils de Lyon, France, during the week of the epidemic peak, the week before and the week after, were selected. Clinical files of the selected patients were retrospectively screened. Pediatric samples (before the age of five) were included for genetic analyses in absence of exclusion criteria (coinfection, comorbidities, treatments or other risk factors). Each patient was also classified depending on the clinical severity of the disease (severe dyspnea, apnea, blood gas alterations, severe radiological signs, oxygenotherapy or hospitalization in intensive care unit). Hypervariable regions of the protein G gene were amplified then sequenced on the Illumina NextSeq 500 platform to analyze phylogenic clustering of severe and mild forms.

## RESULTS

RSV-positive respiratory samples received during three weeks on three consecutive years have been analyzed for a total number of 627 clinical files, distributed on the three years (180, 264 and 183 samples for 2014, 2015 and 2016 epidemics respectively). Most of the RSV-positive population was pediatric (n=537) (429 before the age of one and 96 before the age of five). The phylogenic analysis focused on pediatric samples without exclusion criteria, resulting in 118 clinical files (64% of severe form). Analyses are currently ongoing, showing specific characteristics of each epidemics.

## CONCLUSIONS

Phylogenic analyses are facilitated by the democratization of the NGS technologies. This approach is crucial for an efficient epidemiological surveillance that has to be set up for the next years, reproducing the model of influenza virus surveillance. This need will increase with the development of RSV vaccination.

006

**DELETION IN THE NEURAMINIDASE GENE OF INFLUENZA A VIRUS SUBTYPE H3N2 CONFER ANTIVIRAL RESISTANCE**R. Trebbien<sup>2</sup>, C.B. Christiansen<sup>1</sup>, T.K. Fischer<sup>2</sup><sup>1</sup> Department of Clinical Microbiology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark<sup>2</sup> Virus and Microbiological Special Diagnostics, Statens Serum Institut, Artillerivej 5, Copenhagen S, Denmark**BACKGROUND-AIM**

Antiviral treatment of influenza virus infections can lead to drug resistance of virus. Typically, resistance developed against the most commonly used influenza drug oseltamivir (Tamiflu) is reversible. This study reveals the selection of a deletion in the neuraminidase gene of H3N2 influenza A virus conferring antiviral resistance.

**METHODS**

Respiratory samples from a patient in antiviral treatment were collected before, during, and after treatment and investigated by whole genome sequencing of the influenza virus. A low-frequency-variant analysis was performed using data obtained by next generation sequencing. Neuraminidase-inhibition tests were performed with oseltamivir and zanamivir, and fitness of viruses were evaluated by propagation in sial-transferase gene transfected Madin-Darby Canine Kidney cells.

**RESULTS**

A deletion at amino acid position 245-248 in the neuraminidase gene was induced after initiation of treatment with oseltamivir. The deleted virus had highly reduced inhibition against oseltamivir but was sensitive to zanamivir. Nine days after cease of oseltamivir treatment the deleted H3N2 virus was still present in patient. After three passages of the deleted virus in cell culture, the 245-248 deletion was retained. In the other genes of the influenza viruses, several variants were detected in samples after treatment, most strikingly, was the presence of two major out-of-frame deletions in the polymerase basic 2 gene.

**CONCLUSIONS**

Influenza viruses harboring the 245-248 deletion in the neuraminidase gene, retained fitness after cease of oseltamivir treatment and passages in cell cultures, indicating a potential risk for transmission and circulation of the deleted virus. The neuraminidase gene deletion 245-248 should be included in the future evaluation matrices for antiviral resistance of influenza viruses.

007

**ONGOING MEASLES OUTBREAK IN MILAN: PRELIMINARY DATA OF VIROLOGICAL SURVEILLANCE**A. Amendola<sup>1</sup>, E.R. Frati<sup>1</sup>, S. Bianchi<sup>1</sup>, G. Ciceri<sup>1</sup>, D. Colzani<sup>1</sup>, E. Tanzi<sup>1</sup><sup>1</sup> Department of Biomedical Sciences for Health, University of Milan, Milan, Italy**BACKGROUND-AIM**

Eliminating measles (MV) and rubella (RuV) is a core goal of the WHO Regional Office for Europe (European Vaccine Action Plan 2015–2020). In Italy, the Mo.Ro.Net network, based on a National Reference Laboratory (NRL) and 12 Subnational Reference Laboratory (SRL), was set up for this purpose in March 2017. Since the beginning of 2017, 2,581 cases of MV have been reported in Italy. At the University of Milan, the SRL "EpiSoMI" is responsible for the laboratory confirmation of MV cases and genotypic characterization of the MV circulating during the ongoing outbreak. The present study reports the virological surveillance data of the MV outbreak in Milan and Hinterland.

**METHODS**

The laboratory-based testing for suspected cases of MV were performed at the "EpiSoMI" SRL in Milan (Lombardy Region). Two different samples were analyzed: serum and oropharyngeal swab (UTM™ RT KIT, COPAN ITALIA SpA, Brescia, Italy). Based on the WHO protocol, all biological samples should be collected during the acute phase of the disease, between 4 and 10 days after the exanthema onset. Serological analysis were performed using MV IgM capture Enzyme ImmunoAssay (EIA, Euroimmun AG, Luebeck, Germany). Oropharyngeal swabs were analyzed to detect MV-RNA using RT-PCR targeting the hemagglutinin (H) gene and genotyping by conventional PCR targeting the Nucleoprotein (N) gene.

**RESULTS**

From March 2017, 137 MV suspect cases were investigated and 118 (86.1%; median age: 32 years, range: 2 months-72 years) were laboratory confirmed. Out of 118 confirmed cases, 104 (88.1%) had concordant serological and molecular tests. 11 MV cases (9.3%) were IgM- on serum but RT-PCR+ on oropharyngeal swabs, while 3 (2.6%) cases were IgM+ and RT-PCR-. For 81.8% (9/11) of confirmed cases using molecular assays, samples were collected prematurely (<4 days after the exanthema onset). To date, 82.2% (97/118) of the confirmed cases were successfully genotyped: 86.6% (84/97) were genotype D8 (lineage Hulu Langat) and 13.4% (13/97) genotype B3 (lineage Dublin).

**CONCLUSIONS**

These data indicate that molecular methods using oropharyngeal swabs allows early confirmation and detecting the circulating MV genotypes. High-quality and sensitive virological surveillance is a tool to monitor the outbreak and to achieve the elimination of the disease.

008

# **VIROLOGICAL SURVEILLANCE OF INFLUENZA VIRUSES DURING THE 2016-2017 SEASON AT A TERTIARY UNIVERSITY HOSPITAL IN CATALONIA (SPAIN)**

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## **BACKGROUND-AIM**

The evolution of influenza viruses (FLUV) explains seasonal influenza epidemics and the emergence of resistant variants to antivirals. The aim of this study was to describe the FLUV genetic diversity at our hospital during the 2016-2017 season.

## **METHODS**

From October 2016 to March 2017, respiratory tract specimens were received from patients attended at Vall d'Hebron University Hospital for respiratory viruses infection. FLUV detection was carried out by either immunofluorescent antigen detection or PCR-based assays. A specific real-time one-step RT-PCR assay was performed for influenza A subtyping. The complete coding HA1-domain and the coding neuraminidase protein sequences from severe FLUV cases were sequenced for genetic characterisation and for the screening of amino acid substitutions related to reduced neuraminidase inhibitor drugs susceptibility.

## **RESULTS**

A total of 5,787 specimens from 4,397 cases were studied, of which 960 (17%) samples from 923 (21%) patients were confirmed for FLUV: 698 (76%) influenza A(H3) viruses with 217 (24%) unsubtype influenza A viruses (FLUAV) and 8 (<1%) influenza B viruses (FLUBV). Phylogenetic analysis revealed that 56 (74%) influenza A(H3) viruses belonged to a recent new subclade 3C.2a1 (A/Bolzano/7/2016) and 4 (5%) to the A/HongKong/4801/2013 (3C.2a) phylogenetic group, both with antigenic features like the vaccine strain. A few (16, 21%) influenza A(H3) viruses fell within a novel genetic subset (3C.2a2), with amino acid substitutions likely related to new antigenic features. Two (40%) FLUBV belonged to B/VIC lineage (B/Brisbane/60/2008), while 3 (60%) B/YAM viruses fell to B/Phuket/3073/2013 clade, not included in the trivalent vaccine composition. No mutations in HA associated to virulence (D222N/G) or in NA related to a reduced susceptibility to NAIs were found.

## **CONCLUSIONS**

Most of the viruses detected belonged to genetic groups with similar antigenic features to those of vaccine strains, but a novel subset with likely antigenic differences from the vaccine A(H3) virus emerged. The circulation of a novel subset might be relevant in the next season. Moreover, the co-circulation of viruses belonging to both FLUBV lineages highlights the need for the use of tetravalent influenza vaccine.

009

# **EXPANDING THE SEQUENCING TARGET ENHANCES THE RESOLUTION OF MOLECULAR EPIDEMIOLOGIC STUDIES OF MEASLES VIRUS**

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## **BACKGROUND-AIM**

The genetic characterization of measles viruses is an important tool for measles surveillance as it helps to document chains of transmission, discriminate between imported or indigenous viruses, and monitor progress toward elimination. Measles virus strains are assigned to one of 24 genotypes based on sequence variations in the 450 nucleotides coding for the carboxyl terminal 150 amino acids of the nucleoprotein (N-450). However, in the face of the decreasing diversity of circulating measles genotypes, the resolution provided by N-450 is not always sufficient to distinguish between continued, endemic circulation of the same viral lineage and repeated importations of the same lineage. It will be necessary to expand sequencing targets to increase the resolution of molecular surveillance. To be useful within the Global Measles and Rubella Laboratory Network, (GMRLN) methods for expanding sequence data should be affordable and technically feasible for regional reference laboratories and national laboratories. Whole genome (WGS) of measles virus provides maximum resolution, but is technically challenging. Sanger sequencing of the highly variable non-coding region between the matrix (M) and fusion (F) genes (MF-NCR) may provide increased resolution while using established methods.

## **METHODS**

We have developed primer-independent and amplicon-based library preparation methods for WGS and validated primers for MF-NCR sequencing. We use phylogenetic analysis to compare the ability of WGS and MF-NCR sequences to provide increased resolution.

## **RESULTS**

WGS-t (Whole genome sequences minus the termini, with at least 10-fold coverage) have been obtained from 30 clinical samples and 17 viral isolates. While the majority of samples were genotype B3, samples from six other genotypes were also sequenced, including the first sample of genotype D9. MF-NCR sequences were obtained for 24 of the 28 WHO reference strains. Thirty-nine samples from an outbreak of genotype D9, and 26 samples from an outbreak of genotype H1 were sequenced to measure sequence variability within outbreaks.

## **CONCLUSIONS**

Our data demonstrate that sequencing the MF-NCR, while not providing the same resolution as WGS t, significantly increases the ability to distinguish between measles lineages compared to N-450.

010

# ESTIMATING THE BURDEN OF RESPIRATORY SYNCYTIAL VIRUS IN INFANTS, YOUNG CHILDREN AND THE ELDERLY AT NATIONAL INFLUENZA CENTRE IN SLOVENIA

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## BACKGROUND-AIM

Besides influenza(INF), respiratory syncytial virus(RSV) is a major cause of acute respiratory tract infections(ARI). At National Influenza Centre, Slovenia, data were analysed to estimate the burden of RSV in the most vulnerable age groups(AG).

## METHODS

From weeks 40/2012-20/2017 in total 12732 nasal/throat swabs from patients with ARI, with personal, clinical, epidemiological data were collected from 50 primary healthcare clinics(PHCs) and 2 hospitals(Hs). Nucleic acids were extracted; multiplex-RT-RT-PCRs were used for detection of RSV(RSV-A, RSV-B), INF and other respiratory viruses. AGs were formed: 0-2, 3-6, 7-14, 15-19, 20-64, ≥65 years of age(YA).

## RESULTS

In infants (0-2YA) RSV is leading cause of ARI (21%), followed by INF (9%). In young children (3-6YA) INF is leading cause of ARI (28%), followed by RSV (12%). In AGs 7-14, 15-19, 20-64 YA INF predominates (43%, 44%, 53% respectively), RSV is present only in minor rates (4%, 4%, 4% respectively). In the elderly (≥65YA) INF remains leading cause of ARI (37%), but an increase in RSV is observed (12%). In infants, RSV is detected mostly in those examined in Hs (91%), and less in those examined in PHCs (9%). In AGs 3-6, 7-14, 15-19, 20-64 YA, RSV is detected in similar rates in those examined in PHCs and Hs (40%-60%). In the elderly RSV is detected only in those examined in Hs (100%), suggesting more severe clinical outcomes. In all AGs majority of patients have cough, fever, breathing difficulties (90%-100%, 80%-90%, 40%-60% respectively). Infants more often have bronchiolitis (50%) than pneumonia (6%), the elderly more often have pneumonia (50%) than bronchiolitis (18%). In other AGs bronchiolitis and pneumonia are detected equally in lower rates (10-20%). Acute respiratory distress was observed only in AGs 0-2, 3-6, ≥65 YA (0.4%, 4%, 3% respectively). A plot of all virus detections per week confirms seasonal burden of RSV that often coincides with influenza. In the examined period in Slovenia RSV-A predominated in 2012/2013, 2016/2017; RSV-B predominated in 2013/2014, 2014/2015, in 2015/2016 RSV-A and RSV-B co-circulated. No significant differences in clinical manifestations were observed in regard to circulating type.

## CONCLUSIONS

Results confirm the highest burden of RSV in infants, young children and the elderly.

011

# CHALLENGES OF ENTEROVIRUS SURVEILLANCE APPROACHING THE POST-POLIO ERA AT LABORATORY FOR PUBLIC HEALTH VIROLOGY, SLOVENIA

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## BACKGROUND-AIM

Laboratory for Public Health Virology serves as WHO National Reference Laboratory for Polioviruses and WHO National Influenza Centre for Slovenia. Acute flaccid paralysis (AFP) surveillance is established, but is difficult to perform in a polio-free country. For this reason also specimens for influenza surveillance, collected through a sentinel of 50 primary healthcare clinics(PHCs) and 2 hospitals(Hs), are tested also for enteroviruses (EV) to gain some information of EV circulation in the population.

## METHODS

From 1st April 2015 to 1st April 2017 in total 5405 nasal/throat swabs from patients with acute respiratory infections (ARI), with personal, clinical, epidemiological data were collected. Nucleic acids were extracted; multiplex-RT-RT-PCRs were used for detection of influenza and other respiratory viruses, including EV. Selected specimens, positive for EV, were then sequenced for genotyping.

## RESULTS

In total 243 specimens were positive for EV, out of this 108 were sequenced for genotyping. The majority of EV were attributed to species EV-A and EV-B (55% and 35% respectively). Among species EV-A CV-A6 predominated (47%); CV-A2, CV-A4, CV-A5, CV-A10, CV-A16, EV-A71 were present in lower rates (5%-14%). Among EV-B CV-A9 predominated (24%), followed by CV-B5 and E-9 (both 14%); CV-B2, CV-B3, CV-B4, E-6, E-7, E-13, E-18, E-25, E-30 were present in lower rates (3%-8%). Only 1% of genotyped EV belonged to EV-C (EV-C109) and 8% of genotyped EV belonged to EV-D (all EV-D68). Patients, positive for EV, were more often examined in Hs (87%) than in PHCs (13%). EV were more often detected in infants (0-2 years of age; 69%) and young children (3-6 years of age; 20%) than in older patients. A plot of all virus detections per week confirms a seasonal circulation of EV, usually between April and November, with peaks between July and October.

## CONCLUSIONS

Non-polio EV surveillance based on specimens collected for surveillance of ARI could give useful information on EV circulation in the population, but it is insufficient, as it does not include EV that cause other clinical manifestations (neurological symptoms, HFM disease and other). In this case important information for surveillance of EV circulation and for EV burden determination is missing. A re-design of non-polio EV surveillance is needed in Slovenia.

012

### THE BURDEN OF HUMAN METAPNEUMOVIRUS INFECTIONS IN HOSPITALIZED NORWEGIAN CHILDREN

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#### BACKGROUND-AIM

Human metapneumovirus (HMPV) is a common respiratory virus in children. The burden of severe HMPV respiratory tract infections (RTI) in European children has not been clarified. We studied HMPV, HMPV genotypes and subtypes in Norwegian children and compared hospitalization rates of HMPV and respiratory syncytial virus (RSV).

#### METHODS

During 2006-15, we prospectively enrolled <16 years old children admitted with RTI and asymptomatic controls. Nasopharyngeal aspirates were analyzed by in-house real-time PCR tests for HMPV, RSV and 17 other pathogens. We genotyped HMPV-positive samples, performed a phylogenetic analysis of the F-gene region and assessed HMPV shedding time in 32 children.

#### RESULTS

In children with RTI, HMPV was detected in 267/3,650 (7.3%) and RSV in 1048/3,650 (28.7%). Among controls, 7/339 (2.1%) were HMPV-positive by PCR. The median Ct value of HMPV among children with RTI (28.0, IQR 24.2-32.1) was lower than among controls (38.9, IQR 37.6-39.2) ( $P < .001$ ). In all 117 (44%) of 267 infected children were HMPV culturepositive at admittance compared to none of the controls (0/7). HMPV occurred in winter epidemics of median 5 months duration and spring-summer epidemics (2.5 months). Both HMPV genotypes B ( $n=126$ ) and A ( $n=96$ ), and subtypes B2 ( $n=89$ ), A2b ( $n=80$ ), B1 ( $n=37$ ) and A2a ( $n=12$ ), but not A1 were detected. Both genotypes and at least two subtypes co-circulated each season. Phylogenetic analyses showed that several strains circulated each year, but no clusters or new strains were detected. In children with lower RTI the average annual hospitalization rates were 1.9/1,000 (HMPV) and 10.4/1,000 (RSV). Among children with RTI, median HMPV shedding time by PCR was 13 days (range 6-28 days), but all were culture-negative after 13 days.

#### CONCLUSIONS

HMPV appears in winter and spring-summer epidemics in Norwegian children, with a five times lower hospitalization rate than RSV. Several HMPV strains and subtypes circulate each season. Children become non-infectious from HMPV within 13 days. All asymptomatic controls were culture negative, and only low levels of HMPV-RNA was occasionally detected, indicating a previous infection.

013

### HUMAN CORONAVIRUS IN HOSPITALIZED CHILDREN: A NINE YEAR LONG EPIDEMIOLOGICAL SURVEY FROM NORWAY

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#### BACKGROUND-AIM

Human Coronavirus (HCoV) are known respiratory viruses, but the occurrence and incidence of HCoV among hospitalized children with respiratory tract infections (RTI) has not been determined in a long-term survey.

#### METHODS

From 2006 to 2015 we prospectively enrolled all children admitted with RTI to Children's Department, St. Olavs University Hospital, Norway. Patients were evaluated routinely, and enrolled after informed consent to their caregivers. Nasopharyngeal aspirates (NPAs) from all patients were analyzed, using in-house TaqMan real-time PCR tests for HCoV OC43, NL63, 229E, HKU1 and 15 other pathogens. Virus occurrence was assessed as number of patients with detection of HCoV in NPA.

Hospitalization incidence rates were calculated based on population data from Statistics Norway, ICD10 diagnoses of lower RTI from the hospitals Patient Administrative System and virus occurrence rates from NPAs. Patients were evaluated by a physician and diagnosed with upper RTI (URTI) and/or lower RTI (LRTI).

#### RESULTS

Of 3650 included children, 8.4% ( $n=306$ ) had an HCoV infection. OC43 was detected in 146 children, NL63 in 99, HKU1 in 43 and 229E in 18. The occurrence of all four HCoV varied from season to season. OC43 and NL63 had increased detection rates every second year. During all nine years OC43 was detected in all months, but not every month each year. OC43 was detected prior to NL63 each season. NL63 was never detected from August through October. HKU1 had also high detection rates every second year, but not the same years as OC43 and NL63.

From November 2006 to 2015 the average hospitalization rate of children with LRTI and HCoV infection was 3.90 per 1000 < 2 years of age. The incidence rates varied from 0.97 per 1000 < 2 years of age in the year with lowest detection of HCoV to 7.36 per 1000 < 2 years of age the year with highest number of detection.

#### CONCLUSIONS

Human coronavirus infections are associated with severe RTI in need of hospitalizations. OC43 and NL63 are most common. HKU1 appears frequently whereas 229E is only occasionally detected. OC43, NL63 and HKU1 have seasonal variations and higher detection rates every second year.

014

# RESPIRATORY VIRUSES AND ACUTE RESPIRATORY TRACT INFECTIONS IN CHILDREN UNDER 15 YEARS, MILAN (ITALY) 2015 AND 2016.

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## BACKGROUND-AIM

Acute respiratory tract infections (ARI) are a major cause of children morbidity worldwide. Viruses are the leading etiologies of ARI. We describe the results of molecular detection of respiratory viruses in samples collected from children with ARI admitted to a University and research hospital in Milan (Italy) in 2015 and 2016.

## METHODS

1626 respiratory specimens collected from as many children <15 years with ARI were analysed. After DNA/RNA extraction (EZ1@DSP Kit, Qiagen), samples were tested by a multiplex real-time PCR (Anyplex™ II, RV16-Detection, Seegene) to detect: Adenovirus (AdV), Bocavirus (BoV), Coronavirus (CoV), Parainfluenza virus (PIV), Metapneumovirus (MPV), Enterovirus (EV), Respiratory syncytial virus (RSV), Rhinovirus (RV), and Influenza virus (IV).

## RESULTS

74.9% (1218/1626) of samples were positive for at least one of the viruses included in the panel; 35.8% (436/1218) of these tested positive for more than one virus. RV, RSV and AdV were the targets most frequently detected (51.9%, 26.7% and 15%, respectively), followed by BoV (13.9%), EV (11.8%) and PIV (10.2%). CoV, IV and MPV were the viruses identified less frequently (9.8%, 8.8%, and 5.3%, respectively). AdV and EV were co-detected with other viruses in 79.8% and 68.8%, respectively.

Children who tested positive for at least one virus were younger than those who resulted negative (median age: 13.1 months [IQR: 34.3 months] vs 26.8 months [IQR: 46.36 months];  $p=0.03$ ). Most (57.3%) children with positive sample were <3 years. RSV was detected mainly in infants (median age 4.4 months; [IQR: 15.2 months]) whereas IV in very young children (median age 29.7 months; [IQR: 66.3 months]). 63.2% of positive cases occurred during fall and winter. MPV and EV were detected more frequently in spring and summer.

## CONCLUSIONS

In our ARI series, at least one virus was identified in 75% of cases, with RV and RSV being the main contributors. Viruses were identified in ARI cases throughout the year, particularly in children <3 years. In 1 out of 3 positive-samples more than one virus was detected.

The routine performance of molecular assays to detect a wide range of respiratory viruses can benefit clinical management of patients and can give information on the epidemiology of these viruses.

015

# VIROLOGICAL AND CLINICAL CHARACTERIZATION OF RSV-A, GENOTYPES NA1 AND ON1, AND RSV-B IN INFANTS WITH BRONCHIOLITIS

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## BACKGROUND-AIM

Human respiratory syncytial virus (RSV) is the major cause of bronchiolitis in infants. Different genotypes of RSV-A and -B co-circulate during successive epidemic seasons, with a (not regular) pattern of alternating prevalence. According to most studies, RSV-A causes a more severe bronchiolitis course; however, no significant differences were found in several reports. Inconsistency could be due to the different genotypes circulating in different epidemic seasons because there is still limited data on specific strains.

The aim of this study was to characterize the different RSV genotypes circulating over 11 epidemic seasons and to assess their impact on clinical presentation and disease severity in infants hospitalized for bronchiolitis.

## METHODS

Term infants with no underlying conditions, hospitalized for bronchiolitis in the Paediatric Emergency Department of Sapienza University of Rome during 11 consecutive epidemic seasons (2005/2006-2015/2016) were enrolled. A 0-8 severity score based on respiratory rate, arterial oxygen saturation in room air, retractions and oral feeding ability was determined on admission. Nasal washings were tested for 14 respiratory viruses; RSV-positive samples were sequenced and phylogenetically analyzed. Demographic, familial and clinical data from RSV positive cases were compared and a multinomial logistic regression analysis was performed.

## RESULTS

Of about 300 RSV-positive cases, 272 were successfully sequenced: 133 were RSV-A, genotype NA1; 80 RSV-A, genotype ON1; 59 RSV-B, genotype BA. In the first epidemic seasons, only genotype NA1 was detected among RSV-A cases. In 2011/12, 20% of RSV-A were ON1; afterwards, ON1 completely replaced NA1. All RSV-B belonged to genotype BA. Infants with NA1 had a higher severity score, a higher respiratory rate and a lower oxygen saturation, than infants with the other strains. Infants with RSV-B were older, presented more frequently with fever, and had a family history of asthma and passive smoke more frequently of those with RSV-A.

## CONCLUSIONS

Our results, showing that clinical severity is influenced by RSV genotype and familial risk factors, would be useful to understand bronchiolitis pathogenesis, and plan RSV surveillance and vaccine development.

016

# CHARACTERIZATION OF CLINICAL AND VIRAL KINETICS DURING A NATURAL RESPIRATORY SYNCYTIAL VIRUS INFECTION IN A PRIMARY CARE SETTING IN BELGIUM

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## BACKGROUND-AIM

A better understanding of viral and symptoms kinetics as well as host determinants of Respiratory Syncytial Virus (RSV) disease is important for the clinical development of vaccines and therapeutic agents, and to support the delineation of a therapeutic opportunity window in RSV infected patients.

## METHODS

This prospective longitudinal study targeted patients with an acute respiratory infection (ARI) in a primary care setting. Patients presenting with ARI were screened for RSV during two consecutive RSV seasons (2014-2016). RSV positives were monitored daily as long as symptomatic up to maximum 7 days. RSV viral load (VL) was assessed in mid-turbinate swabs in UTM using qRT-PCR. Clinical recovery was recorded using a Clinical Symptom Score (CSS). Priority for enrollment was given for patients with underlying risk factors for severe RSV disease (@risk).

## RESULTS

Of 239 patients screened, 59 RSV positives were assessed for at least 3 days (primary analysis set). The mean age of these patients was 8.2 y, with ages ranging from 3 months to 66.3 y. The mean duration of symptoms before presenting to the GP was 2.4 days. Twenty-two patients were identified as patients@risk.

The overall mean VL on day 1 was 7.69 log<sub>10</sub> RSV copies/ml. The overall viral clearance rate was 1.4 log<sub>10</sub> RSV copies/ml (VL day1-day3). The VL was not influenced by the risk category, but there was a strong influence of age: the youngest age group (0-<1y) had a higher VL over time than the older children (1-<6y) and adults, and displayed the lowest viral clearance rate (0.56 log<sub>10</sub> RSV copies/ml).

Children 0-<2y had the highest CSS. The 2-<18 y old children had the lowest disease severity, with fast recovery. Patients@risk had a higher CSS throughout the 7 days of assessment than subjects without underlying diseases.

## CONCLUSIONS

In our study, children under 1 y of age have a higher RSV VL and a slow viral clearance rate. Children under 2 y suffer the most from an RSV infection. Nevertheless, the adult population also shows a remarkable RSV VL and disease burden. The RSV disease severity in the patients with underlying risk factors is higher than in patients without risk factor. This disease severity is not driven by RSV VL as we see a comparable or even slightly lower RSV VL in patients@risk.

017

# EVALUATION OF A LYOPHILISED MULTIPLEX CONTROL MATERIAL FOR THE SYNDROMIC DIAGNOSIS OF RESPIRATORY INFECTIONS BY NUCLEIC ACID TECHNOLOGY (NAT)

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## BACKGROUND-AIM

Respiratory diseases represent the largest burden of communicable diseases in developed countries. Their clinical symptomatology could be caused by a large number of aetiological agents causing clinical settings to conduct 'syndromic approach' based on simultaneous testing of respiratory targets.

The National Institute of Biological Standards and Control (NIBSC) produces reference materials designated to aid with compliance of quality systems of clinical laboratories. In order to provide suitable controls for syndromic diagnosis of respiratory diseases, a CE- IVD marked respiratory multiplex working standard for nucleic acid technologies is being developed.

## METHODS

Two lyophilised products, one containing 15 viral and one containing 7 bacterial pathogens were produced. Whole agents were lyophilised in a universal buffer (10mM Tris-HCl - 0.5mM EDTA) and a total of four excipients (trehalose, mannitol, sorbitol and glycerine) were evaluated as stabilisers. Optimal lyophilisation matrix selection was based on post-lyophilization stability of targets at -70°C, 4°C and ambient temperature. ANOVA analysis was employed to establish statistical differences between the lyophilised formulations assessed. Performance of final product was further evaluated against available IVD-CE marked commercial assays.

## RESULTS

Universal formulation of 2% trehalose - 4% mannitol was found to be the most optimal lyophilisation matrix. Stability and degradation studies indicate product stability at ambient temperature for logistics purposes as well as 7-days post-reconstitution at 4°C. Performance evaluation using several multiplex commercial assays indicates that tests detected the targets with similar levels of sensitivity when compared with our in-house assay.

## CONCLUSIONS

The multiplex respiratory reagent represents a cost-effective option as a control for multiplex commercial panels for the diagnosis of respiratory diseases. As the product mimics a clinical sample this makes it suitable as control for extraction and amplification processes, aiding clinical laboratories to comply with their quality control systems. This product complements other multiplex working reagents produced at NIBSC such as the available immunodeficiency panel and the gastrointestinal and meningitis panel currently in development.

018

# ESTABLISH A TEST SYSTEM FOR EVALUATION NEWLY DETECTED MUTATIONS IN RESPIRATORY SYNCYTIAL VIRUS FUSION PROTEIN CONCERNING RESISTANCE TO PALIVIZUMAB

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## BACKGROUND-AIM

Respiratory syncytial virus (2 subgroups A and B) is known as one of the most important respiratory tract pathogen with clinical significance in young children under 2 years of age. There are only two licensed substances allowed in RSV therapy. Ribavirin, a synthetic nucleoside analogue, was approved by FDA for treatment of severe RSV infection in hospitalized children but it has serious side effects at the doses needed to eliminate RSV. Palivizumab, a monoclonal antibody against the RSV fusion protein F, offers passive immunity in prophylactic treatment for high-risk infants. RSV mutants that exhibit resistance to palivizumab have been shown to have amino acid (AA) changes in the antigenic site II (AA 262-275) on the F protein. Therefore it would be desirable to have a stable reverse genetics method for RSV study, that enable to achieve convincing results in RSV characterization testing which in turn can be a building-block in further research with RSV.

## METHODS

The F protein gene sequences of 8 patient isolates (all RSV A) were compared with that of 3 reference strains: line 19, A2 and Long. We introduced identified mutations into the pSynkRSV-line19F BAC (Hotard et al. 2012, BEI Resources) using "en-passant" mutagenesis (Tischer et al. 2006). Reconstructed BAC was then transfected into BSR-T7/5 cells (kindly provided by K. Conzelmann, Munich) with 4 sequence-optimized helper plasmid encoding the RSV N, P, M2-1 and L protein (BEI Resources) for rescue of virus. Recombinant virus from BSR-T7/5 was used to infect Vero cells in order to propagate recovered virus for further characterization step including the growth kinetic, susceptibility testing using plaque reduction and microneutralization assay.

## RESULTS

We identified 7 point mutations and 6 polymorphisms. "En-passant" mutagenesis was successfully conducted for 5 of 7 point mutations. Virions rescued from pSynkRSV-line19F BAC showed significant lower IC50 on Vero cells in comparison to the literature values for palivizumab, which were tested on HEp-2 cell culture.

## CONCLUSIONS

Vero and HEp2-derived RSV induce different infection efficacy due to alterations in the G attachment protein has been shown in previous reports. Whether this effect also leads to a remarkable reduction of IC50 is needed to be clarified with more investigations.

019

# NAKED DNA IMMUNIZATION WITH FULL-LENGTH ATTACHMENT GENE OF HUMAN RESPIRATORY SYNCYTIAL VIRUS INDUCES SAFE AND PROTECTIVE IMMUNE RESPONSE

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## BACKGROUND-AIM

Development of potent vaccine for human respiratory syncytial virus (HRSV) that confers better protection than natural infection remains a global challenge. Vaccination with naked DNA is currently considered successful approach for the control of many viral diseases due to its considerable safety, stability, ease of construction and targeted immunity. In this study, the potential of DNA vaccination using full-length HRSV attachment (G) gene was evaluated in mouse model.

## METHODS

The complete G gene sequence of HRSV type A strain Riyadh 38/2008 was cloned in pcDNA3.1+ vector (pcDNA/GA). The expression potential of pcDNA/GA was confirmed in HEp-2 cells and the immunogenicity was evaluated by testing antibody and cytotoxic T-cell responses in immunized mice. Mice were further challenged by wild-type virus and physiological parameters, clinical signs and mortalities were recorded. Seven days postchallenge, lungs were harvested for evaluation of pulmonary immunopathology using histopathological examination, virus titration and cytokine profiling.

## RESULTS

pcDNA/GA immunized mice exhibited high antibody titers in ELISA with superior neutralization activity, and induced potent HRSV-specific CD8+ T cell response in ELISOPT assay. Following challenge of the immunized mice with the wild-type virus strain, no clinical disease outcomes, no lung viral load, and a significant diminish of pulmonary immunopathology were recorded compared to control mice. The pulmonary cytokine profile in pcDNA/GA immunized mice after challenge displayed notable upregulation of Th1-associated cytokines while that of FIRSV immunized mice exhibited high levels of Th2-associated cytokines.

## CONCLUSIONS

The DNA vaccine candidate pcDNA/GA has proven distinct efficacy and safety in mouse model. Further evaluation in other animal models such as cotton rats and non-human primates is necessary before its use in clinical trials.

020

# **VIRAL RESPIRATORY INFECTIONS DIAGNOSED BY MULTIPLEX PCR IN PEDIATRIC PATIENTS**

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## **BACKGROUND-AIM**

Syndromic diagnosis by multiplex nucleic acid amplification tests (NAAT) is the most practical approach to respiratory tract infections (RTI) since the symptoms are rarely agent specific. The aim of this study was to investigate the respiratory viruses in children admitted to a university hospital with RTI during the last 6 years by a multiplex PCR assay.

## **METHODS**

A total of 2382 respiratory samples collected from children ( $\leq 17$  years) between April 2011 and May 2017 tested by a multiplex real-time PCR assay. Two different commercial assays were used during the study period, "AusDiagnostics/Respiratory Pathogens 12 (Australia)" used between April 2011 and December 2015, which changed to "Fast Track Diagnostics/Respiratory Pathogens 21 (Luxemburg)" after January 2016 in order to cover more viruses. Nucleic acid extraction was done by EZ1 Advanced XL platform (Qiagen).

## **RESULTS**

Respiratory pathogens detected in 1313 of the 2382 (55.1%) samples. The rate of positive specimens were between 41.41 and 55.19 yearly, except 2016 where it increased to almost 70% ( $p < 0.05$ ) due to influenza A in winter months and rhinovirus throughout the year. The most prevalent viruses during the 6-year period were rhino/enterovirus (RV/EV) (34.6%), respiratory syncytial virus (RSV) (22%), and influenza virus A/B (IFV) (12%). RV/EV and adenoviruses detected throughout the year. IFV was most frequently detected during January - March while both RSV and metapneumovirus (MPV) were also in circulation. Bordetella spp, which was detected by AusDiagnostics assay, had low prevalence compared to viruses. Majority of the children with Bordetella spp (16 of 18, 89%) were younger than 3 months of age. The coinfection percentage was 8.9%. Rhinovirus was the most common virus in coinfections while RSV plus rhino/enterovirus were the most frequent combination. Children  $< 5$  years were significantly had more positive results than their older counterparts ( $p < 0.05$ ).

## **CONCLUSIONS**

The most prevalent viruses were RSV, RV/EV and IFV. The rate of positive samples changed between 40 to 55% yearly. There was a significant increase in IFV A and RV positivity in 2016. RSV and hMPV showed a similar seasonal distribution to IFV, which made it necessary to use a virological diagnostic assay.

021

# **MOLECULAR EPIDEMIOLOGY OF HUMAN MASTADENOVIRUS DETECTED IN RESPIRATORY SPECIMENS AT A TERTIARY CARE UNIVERSITY HOSPITAL IN CATALONIA (SPAIN) DURING THE 2013-2015 SEASONS.**

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## **BACKGROUND-AIM**

Human mastadenoviruses (HAdVs) are divided into 7 species (A to G), which include over 70 genotypes. HAdV-B, -C and -E species are usually associated with respiratory disease. Loop 1 (L1) and C-terminal region of the hexon gene have been widely used for genotyping. In the present study, epidemiological features and viral diversity of HAdV-positive cases detected at Hospital Universitari Vall d'Hebron (Barcelona, Spain) have been described.

## **METHODS**

From April 2013 to December 2015 respiratory specimens from patients with respiratory disease were received and laboratory-confirmed for HAdVs by using an antigen-detection direct immunofluorescence or a real-time multiplex RT-PCR assays. Both L1 and C-terminal regions were amplified and sequenced from HAdVs laboratory-confirmed samples for NJ phylogenetic analyses of HAdV sequences in MEGA v5.2.

## **RESULTS**

A total of 13,582 samples were collected, of which 315 (2%) from 277 (4%) patients were HAdV confirmed. A total of 105 (32%) samples were co-detected with rhinoviruses (54%) and human respiratory syncytial viruses (19%). Regarding the age group distribution, 65% were younger than 4 years, 19% between 5 and 14 years, 11% between 15 and 64 years, and 5% older than 65 years. HAdV detection was variable along the study period, and a seasonality pattern could not be established. Both L1 and C-terminal amplification were successfully for 133 (42%) strains, but for 70 (22%) strains only one region could be amplified. Among the four detected species (B, C, D and E), up to 9 genotypes could be distinguished [HAdV-B3 (64, 32%), HAdV-B7 (3, 1%), HAdV-B14 (1,  $< 1\%$ ), HAdV-C1 (26, 13%), HAdV-C5 (26, 13%), HAdV-C2 (46, 23%), HAdV-D20 (1,  $> 1\%$ ), HAdV-D8 (5, 2%) and HAdV-E4 (11, 5%)]. A correspondence in the taxonomic classification within the 9 genotypes were accomplished by both L1 and C-terminal regions. However, 46 C-terminal region sequences clustered together with HAdV-C2 and HAdV-C6 reference sequences, and were then classified using L1 phylogenetic result.

## **CONCLUSIONS**

The present study reports recent data about genetic diversity of HAdV detected in attended patients at a tertiary hospital in Barcelona, Spain. Phylogenetic analysis of C-terminal region and L1 sequences and their correspondence contribute for an accurate classification of HAdV.

022

### IMPLEMENTATION OF THE BIOFIRE FILMARRAY RESPIRATORY PANEL 2 PLUS AS A SYNDROMIC APPROACH FOR DIAGNOSIS OF RESPIRATORY INFECTIONS.

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#### BACKGROUND-AIM

We implemented the BioFire FilmArray Respiratory Panel 2 plus (RP2plus) assay (RUO version) in order to compare its analytical performance with our standard of care (SOC) PCR technique (Respiratory panel MWS r-gene, Argene-bioMerieux) and define the added value of this complete panel including 22 respiratory pathogens (18 viruses, 4 fastidious bacteria).

#### METHODS

We tested fresh nasopharyngeal swab clinical samples from 205 patients suspected of respiratory infection (83 female, 122 male, 38.54 mean age [0.5 to 98]). 27 were from the emergency department, 163 inpatients and 15 were outpatients. Samples were prospectively collected during 2 months (from 20th of September to 17th of November 2016) and prospectively tested in parallel with the RP2plus assay (third version of the RP assay) and with our SOC routine test (MWS r-gene) for which the tests were performed according to viral epidemiology (picornavirus detection) and/or specific clinical requests.

#### RESULTS

37 (18.05%) concordant results:

- 1) 31 Picornavirus
- 2) 2 influenza A
- 3) 2 Respiratory syncytial virus
- 4) 2 Mycoplasma pneumoniae

23 (11.22%) discrepant results (FA+ SOC -):

- 1) 22 Picornavirus
- 2) 1 Chlamydia pneumoniae

2 discrepant results (FA- SOC +):

- 1) 2 Picornavirus

33 (16.1%) positive with FA, not tested by SOC procedure:

- 1) 17 Parainfluenza virus (10 PIV3, 6 PIV4 and 1 PIV2)
- 2) 6 Adenovirus
- 3) 6 Coronavirus (2: 229E and 4: OC43)
- 4) 3 Influenza A H3N2
- 5) 1 Mycoplasma pneumoniae

Positivity rate was 19% with our SOC (39/205) and 45% with RP2plus panel (93/205), corresponding to +18.5% pathogen detection.

In addition, considering only the pathogen tested in our SOC, RP2plus panel also increased the positivity rate from 19 to 29.3% (60/205), showing better performances than our SOC, especially for picornavirus detection.

#### CONCLUSIONS

The RP2plus panel showed excellent performances for viruses and fastidious bacteria detection. The added value compared to our SOC is clear and could be valuable in critical clinical situations requiring only 45 minutes for testing 22 respiratory pathogens on 300 µL of nasopharyngeal swab samples, the FilmArray RP2plus is an easy (2 mins of hands-on time) and rapid tool for 24 hours / 7 days respiratory diagnosis.

023

### RESPIRATORY SYNCYTIAL VIRUS (RSV) VIRAL LOAD AND CLINICAL SYMPTOMS DURING FOUR CONSECUTIVE RSV-SEASONS IN A PRIMARY CARE SETTING IN BELGIUM.

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#### BACKGROUND-AIM

Human Respiratory Syncytial Virus (RSV) disease has a high incidence and medical burden in children under 5 years of age. In most adults, RSV would not be differentiated from the myriad of other viral agents causing upper respiratory tract infections (URTI). However, data suggests that RSV may engender an appreciable proportion of the more prolonged URTI and contribute to infections with bronchitic symptoms and wheezing, resulting in many outpatient medical visits and work absence. This primary care setting will accommodate most of the pediatric and adult/elderly RSV cases during the RSV epidemic season.

#### METHODS

Four hundred sixty nine patients diagnosed with an acute respiratory infection (ARI) were screened for RSV during four consecutive RSV-seasons (2012- 2016) in Belgium. RSV Viral load (VL) was determined in mid-turbinate swabs (Copan) using qRT-PCR. Disease severity was assessed using a clinical symptom score (CSS).

#### RESULTS

During the RSV epidemics, 42.6% of patients presenting with ARI were RSV positive. Most of the RSV positives (75.5%) were children (≤6y), with 24.5% younger than one year old. The adult and elderly (>18y) represented 20% of the RSV positives. The mean reported duration of symptoms before GP visit was 2.7 days. The highest mean VL was observed for the youngest group (<1y): 8 log<sub>10</sub> RSV RNA copies/ml and the lowest mean VL was seen in the adult population (18-60y) with 6.0 log<sub>10</sub> RSV RNA copies/ml. In the age groups 0-1y, 1-2y and ≥ 60y, a higher mean and median CSS are observed for the RSV infected subjects in comparison to RSV negative patients. The mean oxygen saturation level in RSV-infected children increased over the different age categories with increasing age. For the elderly population (≥ 60y) a decreased oxygen saturation level of 95.8% was observed.

#### CONCLUSIONS

This study provides evidence of the RSV disease burden in primary care, regardless of age. During the RSV season, 42.6% of patients presenting with ARI were infected with RSV. Though most of the patients were children ≤ 6y (75.5%), one out of five patients were adults (including elderly), indicating a so far neglected RSV disease burden in the adult population.

024

# **HUMAN METAPNEUMOVIRUS FREQUENCY AMONG ADULTS PRESENTING WITH ACUTE RESPIRATORY INFECTION IN TOULOUSE (SOUTH WESTERN-FRANCE) DURING A THREE-YEAR PERIOD**

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## **BACKGROUND-AIM**

Human metapneumovirus (hMPV) is a paramyxovirus that causes both upper and lower respiratory tract infections. Here we report the frequency of hMPV acute infections among adults between 2014 and 2017.

## **METHODS**

Retrospective data from respiratory samples were recovered from the Laboratory Informatics System during three periods: period 1: May 2014 to April 2015; period 2: May 2015 to April 2016 and period 3: May 2016 to April 2017.

All samples had been tested with the Anyplex RV16 respiratory panel® (Seegene) that can detect 16 viruses: influenza viruses types A and B, parainfluenza viruses 1 to 4, respiratory syncytial viruses A and B, rhinovirus, coronaviruses 229E, OC43 and NL63, human metapneumovirus, bocavirus, enterovirus and adenovirus.

## **RESULTS**

3079, 4062 and 4357 samples were tested during the three-year period and 1501 (48.7%), 1866 (45.9%), 1996 (45.8%) samples tested positive for at least one virus of the panel.

hMPV was detected in 85 (5.7 %), 93 (5%) and 124 (6.2%) samples and was detected specifically among adults in 21 (1.4%), 27 (1.4%) and 48 (2.4%) samples (i.e. 21, 20 and 42 patients).

Sex ratio F/H was 2.5, 0.7 and 1.6 during the three-year period.

Mean age was 54.9, 57.5 and 63.8 among patients presenting with hMPV infection and was 53.7, 48.7 and 58.9 among adults infected with other respiratory viruses ( $p=0.775$ ,  $0.027$  and  $0.129$  respectively).

hMPV was detected in mono infections in 81, 96.6 and 89.6%. Multi-infections were always bi-infections with Flu A (1) RSV-A (2), RSV-B (2) adenovirus (1) rhinovirus (3) and coronavirus NL63 (1).

hMPV was most frequently detected during the winter months (November to March). Apart from this period, 6 samples only were positive (May and June).

7 (33.4%), 6 (30%) and 12 (28.6%) patients were hospitalized in intensive care units with severe respiratory manifestations. Only one patient presenting with haematological disease showed prolonged excretion (13 days).

## **CONCLUSIONS**

During the past three years, rates of hMPV respiratory infection remained stable and among adults in particular, the rate was very low. hMPV infection was very rarely observed apart from the winter months and was most often observed in mono-infection. One-third of adults showed severe infection all along the three-year period.

025

# **DETECTION AND CHARACTERIZATION OF RESPIRATORY SYNCYTIAL VIRUS ON1 GENOTYPE INFECTIONS IN CENTRAL MEXICO, 2009-2014**

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## **BACKGROUND-AIM**

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections in young children and vulnerable adults. RSV isolates are classified into two antigenically distinct groups, A and B. In 2012, a novel RSV A genotype, named ON1, was detected in Canada. The ON1 genotype, which contains a 72 nt duplication in the C-terminal region of the G gene sequence, has been reported to circulate in many countries since then. The objective of this study was to identify and characterize the presence of RSV ON1 genotype infections in Mexican children with acute respiratory infections.

## **METHODS**

The study included children <5 years of age hospitalized with acute respiratory tract infections in San Luis Potosí (Central Mexico) between May 2003 and December 2014. Over the study period there were 1153 patients with confirmed RSV infection. We randomly selected 30% of the samples from this patients ( $n = 345$  samples) to determine the type (A or B) and to characterized RSV A viruses (as ON1 or non-ON1) with the use of two RT-PCR protocols. Sequencing of the G gene of a subset of these viruses was carried out.

## **RESULTS**

Among the 345 samples included in the study, 216 corresponded to RSV A and 142 to RSV B (including 13 RSV A and RSV B coinfections). Among the RSV A cases, 106 were caused by the ON1 genotype (including 9 cases with RSV B coinfection). No significant differences in the clinical characteristics between those with ON1 and non ON1 infections were observed. The earliest case of ON1 infection was detected in 2009, almost one year prior to the oldest strain reported to date. ON1 RSV was detected in 13 of the 35 samples (37.1 %) from patients admitted during the 2009-2010 winter season. In the 2011-2012 and 2012-2013 seasons the majority (81 out of 97 samples tested, 83.5 %) were caused by the ON1 genotype.

## **CONCLUSIONS**

Circulation of RSV ON1 genotype was detected in Central Mexico starting in 2009. As far as we are aware, the strains identified in this study include the oldest isolate of RSV A ON1 detected to date. The characterization of the genomic and epidemiological features of RSV viruses in our region provides relevant information for the understanding of the emergence of this new viral genotype.

026

### OUTBREAK REPORT OF NOSOCOMIAL TRANSMISSION OF INFLUENZA B VIRUS IN A MINOR EMERGENCY HOSPITAL IN WESTERN SWEDEN 2016

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#### BACKGROUND-AIM

To describe a hospital outbreak of influenza B by combining molecular methods with clinical data.

#### METHODS

Laboratory testing with multiplex real-time PCR of nasopharyngeal swabs (NPS) confirmed a total of 20 cases of influenza B diagnosed at Kungälv hospital between 5th and 23th of May 2016. Medical records were reviewed for patient characteristics, exposure and outcome. Timelines for development of clinical symptoms, NPS sampling, PCR confirmation, and antiviral treatment were constructed. 18 of the influenza B samples underwent genome sequencing and phylogenetic analysis. Medical records of all patients with a positive NPS for influenza at the local laboratory during an extended time period were reviewed to find possible connections to the outbreak. Furthermore, records were also reviewed of all patients admitted to the affected ward to find undiagnosed cases and to evaluate the extent of antiviral prophylaxis being used.

#### RESULTS

All 20 cases of influenza B were of subtype B/Yamagata. 17/20 patients (index included) could be linked to each other by transmission in one single hospital ward. In 15/17 cases, phylogenetic analysis was possible and could support a close relationship between the strains. 17/75 patients admitted to the affected ward during the outbreak period were diagnosed with influenza resulting in an attack rate of 23%. 9/75 patients (12%) were given antiviral prophylaxis to prevent infection. Only one patient was diagnosed with influenza B despite prophylactic treatment. The mean length of hospital stay were 17.1 days, and patients diagnosed with influenza B were submitted to a total of 14 readmissions. One probable case of influenza-related death was found. 16/20 cases could be classified as hospital acquired infections. The PCR-findings of influenza B represents 65 % (n= 20/31) of the total positive findings of influenza B at the local laboratory during the outbreak period.

#### CONCLUSIONS

We found that influenza B infection may efficiently spread in a minor emergency hospital. We believe early identification of probable cases, rapid diagnosis and means to identify and predict transmission events combined with early recognition of suspected outbreaks can be important factors in controlling nosocomial influenza.

027

### MULTICENTER CLINICAL EVALUATION OF THE IDYLLA RESPIRATORY (IFV-RSV) PANEL\* USING NASAL AND NASOPHARYNGEAL SWAB OBTAINED FROM SYMPTOMATIC PATIENTS

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#### BACKGROUND-AIM

Rapid and accurate diagnosis of respiratory infection provides opportunity to improve patient management, initiate antiviral therapy and reduce improper use of antibiotics. Idylla™ Respiratory (IFV-RSV) Panel (Janssen Pharmaceutica NV, Belgium) run on the Idylla™ system (Biocartis NV, Belgium) is an automated, multiplex PCR assay that simultaneously detects and reports Influenza A (seasonal H1, 2009 pandemic H1, seasonal H3 and oseltamivir resistant, 2009 H1 mutant H275Y variant), Influenza B and respiratory syncytial virus (RSV). The aim of this study was to evaluate the performance characteristics of IFV-RSV Panel using direct nasal swab (NS) and nasopharyngeal swab (NP) eluted in viral transport media (VTM).

#### METHODS

This multicenter study included prospective enrollment: i) from 4 USA sites during 2015-2016 and collection of 214 paired samples (NS and NP), ii) 800 paired samples previously collected from sites (6 in USA and 1 in Belgium) during 2012-2014 and archived as frozen aliquots. NS were tested directly by placing into the Idylla cartridges. For NP, 200 µl of VTM was added into each cartridge and tested. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of IFV-RSV Panel was determined by comparing to a FDA-cleared multiplex assay, Verigene Respiratory Virus Plus Nucleic Acid test-RV+ (Luminex Inc., TX). Bi-directional sequencing (LabCorp, US) was used for discrepant analysis.

#### RESULTS

From the 1014 subjects (children=740, adult=274) enrolled, 935 NS and 960 NP200 met all criteria and were included in final data analysis. The PPA for Idylla IFV-RSV Panel for FluA, FluB and RSV with NS samples was 92%, 85%, 89% and 92%, 86%, 87% with NP samples respectively. The NPA for Idylla IFV-RSV Panel for FluA, FluB and RSV for both NS and NP samples was > 99%. Following discrepant resolution by sequencing, PPA increased to >95% (Flu A, B) and >90% (RSV) for both sample types.

#### CONCLUSIONS

Idylla IFV-RSV Panel is an ideal, fully automated, multiplex molecular assay capable of detecting Flu A, Flu B and RSV in symptomatic patients within 1 hour. Direct NS or NP specimen eluted in VTM are preferable sample types for Idylla IFV-RSV Panel assay.

\*developmental product, sample types may not be reflected in final product

028

### LOW DETECTION RATE OF VIRAL RESPIRATORY PATHOGENS IN ASYMPTOMATIC ADULTS

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#### BACKGROUND-AIM

Modern PCR techniques for detection of respiratory pathogens in the upper airways are highly sensitive and interpreting the clinical relevance of a positive result can be challenging. We therefore conducted a prospective study to investigate the detection rate of pathogens causing respiratory tract infections (RTI) in asymptomatic adults.

#### METHODS

A prospective case-control study collecting naso-pharyngeal (NP) swab samples from adults with and without symptoms of RTI during 12 consecutive months at primary care centres and hospital emergency inpatient wards in Western Sweden. Clinical and laboratory data were recorded. All included controls reported absence of symptoms of RTI, fever and diarrhoea two weeks prior and four days post enrolment. Swabs were analysed for detection of respiratory pathogens in a multiplex real-time PCR assay targeting sixteen viruses and four bacteria.

#### RESULTS

444 controls and 103 cases were included. A virus was detected in 4.3% of the controls included in primary health care compared to 4.1% in the hospital setting. In total 35% of cases were positive for a virus (44.4% in primary care). Human rhinovirus (HRV) was detected in 3.2% of the controls compared to 21.4% in cases ( $p < 0.01$ ). *Streptococcus pneumoniae* was equally common in both groups (5.9% and 5.8% respectively). Factors associated with detection of either viruses or bacteria were analysed through a multivariate logistic regression model and independently associated with viral detection in asymptomatic subjects were: age  $> 65$  ( $p = 0.03$ ). Current smoking and co-morbidity were identified as variables that significantly increased the detection rate of bacteria ( $p < 0.01$ ).

#### CONCLUSIONS

Detection rate of respiratory viruses in adult asymptomatic individuals was low and HRV was the most prevalent finding in the asymptomatic group. Age  $> 65$  and comorbidity was associated with an increased detection rate of virus in controls. False positive detection of respiratory viral pathogens is unlikely when multiplex-PCR methods for respiratory pathogens are used for diagnostic purposes in adult patients.

029

### PARAINFLUENZA VIRUS 4 IN SLOVENE CHILDREN

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#### BACKGROUND-AIM

Parainfluenza virus 4 was first described in 1960. PIV 4 is rarely routinely detected and a few studies showed that PIV 4 is mainly responsible for the milder respiratory infections. The PIV 4 pathogenicity is underestimated since the virus is rarely routinely detected and with only a few epidemiological studies and clinical cases of respiratory infections, published. Development and improvement of specific RT-PCR for the detection of PIV 4 offers new possibilities for detection. Those techniques are faster, more specific and sensitive in comparison with conventional diagnostic techniques. New data due to the wider use of advanced diagnostics indicate a higher prevalence of PIV 4, as we expected so far.

#### METHODS

In 2015 Slovenian retrospective cohort study was conducted, in which 2474 children under 6 years of age were included to assess the importance of PIV 4 infections in pediatric patients. All of the children were routinely tested for respiratory viruses which are included in in-house developed respiratory panel, which includes the detection of influenza A and B virus (FluA/B), respiratory syncytial virus (RSV), rinoviruses (hRV), human metapneumovirus (hMPV), coronaviruses (HCoV-NL63, OC43, HKU1 and 229E), human Bocavirus 1 (HBoV1), adenoviruses (AdV), parainfluenza virus 1-3 and enteroviruses. We detected PIV-4 using in-house developed real-time RT-PCR retrospectively for the purpose of the present study.

#### RESULTS

PIV 4 was detected as a single respiratory virus in 40 children (1.6%) with respiratory infections and that accounted for 33.9% of all infections caused by PIV 1-4 in 2015. PIV 4 was most frequently (3.1%) detected in children between five and six years of age. The seasonal distribution of PIV 4 infections with the peak in August 2015 was also detected. Acute upper respiratory infection (45%) and pneumonia (22.5%) were the most commonly diagnosed. Supplemental oxygen for respiratory insufficiency was given to 27.5% of children and 60% of children had an associated underlying disease.

#### CONCLUSIONS

The results of our study showed, that PIV 4 is an important respiratory pathogen, therefore we included the virus into routine diagnostics in so called respiratory panel.

030

**GENETIC DIVERSITY OF ENTEROVIRUS IN BELGIUM IN 2016**E. Wollants<sup>2</sup>, J. Vercruyssen<sup>2</sup>, M. Van Ranst<sup>1</sup><sup>1</sup> Department of Laboratory Medicine, University Hospitals Leuven<sup>2</sup> Laboratory of Clinical Virology, Rega Institute for Medical Research, KU Leuven**BACKGROUND-AIM**

Human enteroviruses infect millions of people worldwide each year. Some infections are asymptomatic but enteroviruses have been associated with a wide spectrum of common and uncommon illnesses, such as common cold, acute hemorrhagic conjunctivitis, myocarditis and poliomyelitis. Enterovirus B are the most common cause of aseptic meningitis worldwide. This mainly affects young children and in most cases requires hospitalization. Enteroviral meningitis often appears in the form of outbreaks and peaks during the summer and early fall. Enterovirus D68 (EVD68) is associated with severe respiratory illness and neurological complications and EVA71 is a common causative agent of hand foot and mouth disease. We investigated which enterovirus genotypes were circulating in Belgium in 2016.

**METHODS**

Positive enterovirus samples were collected from the University Hospital Gasthuisberg, Leuven, Belgium from January until December 2016. Different sample types such as cerebrospinal fluid, feces, pharyngeal swabs and aspirates were used for the initial diagnosis of the enterovirus infection. Molecular typing was done by RT-PCR on these clinical samples, followed by sequencing part of the gene coding for the VP1 capsid protein, using different primer sets.

**RESULTS**

Based on a fragment in the VP1, 187 samples could be assigned to different genotypes: echovirus 30 (55.6%), echovirus 5 (7%), coxsackievirus B4 (5.3%), coxsackievirus B5 (4.8%). Sixteen other genotypes were only sporadically detected in our sample collection (< 4%). We found 9 patients with EVD68 (4.8%) and 3 patients with EVA71 (1.6%).

**CONCLUSIONS**

For epidemiological surveillance and to study enterovirus evolution, all positive enterovirus samples collected in a large Belgian hospital in 2016 were analyzed. They were molecularly typed using VP1 RT-PCR for enterovirus.

Twenty different enterovirus genotypes were found in cerebral fluid, respiratory samples and feces. The predominant enterovirus type identified in this study was echovirus 30. This is one of the enteroviruses isolated most frequently and has been increasingly prominent in outbreaks since its characterization. The more pathogenic genotypes EVD68 and EVA71 were only detected in a small number of samples.

031

**SEASONALITY AND VIRUS DETECTION RATES OF 16 RESPIRATORY VIRUSES IN SYMPTOMATIC CHILDREN AND ADULTS**X.E. Chen<sup>1</sup>, K.S. Chan<sup>1</sup>, L.E.L. Oon<sup>1</sup><sup>1</sup>Molecular Laboratory, Department of Molecular Pathology, Singapore General Hospital, Singapore 169856**BACKGROUND-AIM**

Respiratory tract infections (RTIs) are major infectious diseases of upper and lower respiratory tract and are caused mainly by viruses and occasionally by bacteria. Early identification of the etiology of a RTI is critical for disease management. A retrospective study was performed for year 2016 to investigate the viral etiology of patients with RTIs in a tertiary hospital in Singapore. Detection rates and incidence of various viruses were compared between children ( $\leq 5$  years old) and adults over the years. Seasonality of viral RTIs was also described.

**METHODS**

Respiratory samples from both inpatients and outpatients received at the hospital's molecular diagnostic laboratory for one year from 1 January to 31 December 2016 were included in the study. Seegene Multiplex real time PCR (Anyplex II RV16 Detection, version 1.1, Seegene, South Korea) was used for the simultaneous detection of 16 viruses for all samples.

**RESULTS**

Over a one year period from January 2016 to December 2016, a total of 9,582 samples were sent for respiratory virus detection, of which 3,731 samples (38.9%) were positive for at least one of the 16 viruses included in the assay. Co-infection with 2-5 viruses was detected in 331 samples, which accounted for 8.9% (331/3,731) positive samples. The monthly positive rates ranged from 32.3% to 45.5%, with a mean of 39.0% (standard deviation 4%). The top 5 viruses detected for children  $\leq 5$  years old were Rhinovirus (25.3%), Adenovirus (16.8%), Bocavirus (8.9%), respiratory syncytial virus (RSV) B (8.0%) and RSV A (7.9%), whereas Influenza (with 24.7% type A and 9.3% type B) were the most common virus etiology for patients  $> 5$  years old, followed by Rhinovirus (23.9%) and Adenovirus (10.4%). Most of the major causative viruses were detected over the whole year period with a seasonal peak observed for Influenza A in June to August.

**CONCLUSIONS**

Viruses were detected in more than one third of respiratory tract infection patients in this study. The most common viruses detected were Rhinovirus and Influenza (A & B) virus. Rhinovirus predominates in children  $\leq 5$  years old, whereas the most common cause of viral RTI was Influenza (A & B), in patients more than 5 years old with Influenza A peaks between June and August.

032

**MEASLES VIRUS AND GENETIC CHARACTERIZATION DURING AN OUTBREAK**J. Dina<sup>1</sup>, A. Vabret<sup>1</sup>

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**BACKGROUND-AIM**

Measles virus genotyping in association with epidemiology data is recommended by the World Health Organization (WHO) for measles surveillance and outbreak control. Genotyping is based on a 450 nucleotides fragment analysis situated at the carboxy-terminal end of the gene coding for the nucleoprotein (N-450). The complete H gene characterization is required only when a new genotype is suspected.

Outbreaks are frequently characterized by the circulation of one of the 24 measles virus genotype. The variability of the N-450 fragment is very low and lead to a difficult intra-epidemic measles characterization

The aim of this study was to analyze and compare molecular epidemiology of N-450 fragment and measles virus genes coding for phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin (H) and the longest non-coding region situated between M and F genes (1012 nucleotides).

**METHODS**

One hundred D4 measles viruses sharing identical N-450 fragment and circulating during the 2009-2011 measles outbreak in France were analyzed.

Amplification and sequencing of complete P, M, F and H genes were performed. Also, the non-coding region situated between M and F genes was completely sequenced and analyzed.

Variability was studied after alignments with Bioedit software. Phylogenetic analyses were performed using Mega 6 software. The tree resolutions were compared.

**RESULTS**

A total of 27 measles strains were analyzed for complete P, M, F, H genes and the M/F non-coding region. The comparison of trees obtained after phylogenetic analyses showed that M/F NCR sequence provides a good phylogenetic tree resolution.

**CONCLUSIONS**

The M/F non-coding region fragment analysis could give complementary epidemiologic information to classical genotype characterization after N 450 terminal sequencing.

033

**MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS (MERS-COV): SURVEILLANCE AND TESTING IN THE NORTH WEST OF ENGLAND**H.Z. Farooq<sup>1</sup>, N. Machin<sup>1</sup>, A.J. Turner<sup>1</sup>, M. Guiver<sup>1</sup>, L. Hesketh<sup>1</sup>, E. Davies<sup>1</sup>, S. Ahmad<sup>1</sup>

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**BACKGROUND-AIM**

A novel coronavirus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) emerged in Saudi Arabia in 2012 and has caused an epidemic in the Middle East. With an imported case in the UK in 2012 and further cases worldwide, it is essential to remain vigilant in preventing the spread of MERS.

Public Health England (PHE) Manchester laboratory is one of the three PHE centres in the UK that perform testing for MERS CoV. The Greater Manchester (GM) region has a population of around 2.5 million people, some of whom travel to the Middle East for various purposes. Manchester also has the UK's 3rd busiest airport with a significant proportion of passengers returning from the Middle East. This results in a high number of travelers returning to the GM region being evaluated for MERS. The results of the PHE Manchester MERS surveillance from 2012 to 2017 is presented in this report.

**METHODS**

Retrospective data was collected for returning travellers from the Middle East fitting the PHE MERS-CoV case definition and presenting with respiratory symptoms in the GM region. This was collected from the initial period of 2012 to 2013 and then from 2015-2017 when testing was re-started. Respiratory samples (N&T swabs, sputum or BAL) were tested for respiratory viruses and MERS CoV using an in-house RT-PCR assay.

**RESULTS**

187 samples from 112 patients that fitted the case definition were tested for MERS and respiratory viruses. No MERS CoV infections were identified by PCR. 62% of samples were PCR positive for viral or bacterial pathogen with Influenza A being the predominant virus (50%). Due to the high sensitivity of the testing, 72% of total patients had a pathogen identified. Peak testing occurred after the Hajj and vacation periods. The majority of patients had travelled from Saudi Arabia (56) and patients with multiple samples demonstrated a 100% diagnostic yield compared to 64% when one sample per patient was sent.

**CONCLUSIONS**

Although no cases of MERS CoV were identified, the majority of patients had Influenza infection for which Oseltamivir treatment was indicated and for whom isolation was warranted. Despite a lack of imported UK cases, it is of the utmost importance that we continue active surveillance; rapidly identifying and isolating potential cases. Only by remaining vigilant can we halt the spread of MERS.

034

# **GENOTYPISATION OF ADENOVIRUS IN SIMULTANEOUSLY COLLECTED NASOPHARYNGEAL SWABS AND STOOL SAMPLES FROM CHILDREN HOSPITALIZED FOR ACUTE BRONCHIOLITIS, ACUTE GASTROENTERITIS, AND FEBRILE SEIZURES**

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## **BACKGROUND-AIM**

Human adenoviruses (HAdV) are divided in 7 species designated with letters A through G, and more than 50 recognized serotypes. Most common genotypes found in patients with respiratory infection are from species C, genotypes 1, 2, 5 and 6, and occasionally from species B, genotypes 3 and 7. In young children, HAdV infections can cause also gastrointestinal symptoms even though, their primary site of infection is respiratory tract. Two genotypes of HAdVs have been associated with diarrhea, F40 and F41, other, so called nonenteric HAdVs were also detected. Genotyping of HAdVs is based on hyper variable regions of the hexon gene, where antigenic domains have been mapped.

## **METHODS**

The study presented here was part of a 2-year prospective study on viral respiratory and gastrointestinal infections in children under 6 years of age from October 2009 to September 2011. Children hospitalized with acute bronchiolitis (AB), acute gastroenteritis (AGE), febrile seizures (FS) and healthy control group were included in our study. Nasopharyngeal (NP) swabs and stool samples were taken from patients and healthy controls. Total nucleic acid from clinical samples was isolated with MagNA pure compact instrument according to manufactural instructions. HAdV was detected by real-time PCR performed on hexon gene (132 bp). For identification of genotype, positive HAdV samples had their hexon gene (956 bp) amplified and sequenced on ABI 3500.

## **RESULTS**

HAdVs were detected in 107/718 (14.9%) NP swabs and in 189/629 (30.0%) stool samples from patients and in 15/156 (9.6%) NP swabs and 40/150 (26.7%) stool samples in healthy controls. In children with AGE (70/218, 32.1% and 44/218, 20.2%) and FS (52/165, 31.5% and 30/192, 15.6%) was a higher probability of detection of HAdV compared to those with AB (23/246, 9.3% and 33/308, 10.7%) in stool and NP samples, respectively. Simultaneously detected HAdVs in both NP and stool sample were in 79/629 (12.6%) patients. 131 isolates were suitable for possible sequence analysis (Ct value 30 or lower) and 71 were successfully characterized, 49 from stool and 22 from NP swabs.

## **CONCLUSIONS**

The most common genotypes in NP swabs were C2 (9/22, 40.9%) and C1 (6/22, 27.3%). The most common genotypes in stool samples were C2 (16/49, 32.6%) and F41 (16/49, 32.6%).

035

# **IMPLEMENTATION OF A RAPID INFLUENZA PCR ALGORITHM AT THE EMERGENCY DEPARTMENT OF A LARGE UNIVERSITY HOSPITAL**

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## **BACKGROUND-AIM**

During influenza season rapid and reliable identification of patients with influenza in the hospital is important to prevent nosocomial spread and to start antiviral therapy in patients with underlying diseases. Rapid tests for antigen detection are easy to use but have a low sensitivity in adult patients and may have sensitivity problems with new virus variants. Novel rapid molecular tests for the detection of influenza viruses are now available. These assays are usually performed in the lab where experiences with molecular assays and the statutory quality assurance steps are present. However, the samples have to be transported to the laboratory and 24h service is often not available. As a consequence, the results are available only after several hours.

## **METHODS**

The Institute for Virology implemented a Cepheid Gene Xpert IV at the emergency department (ED) of the university hospital in Freiburg, Germany. The ED staff was instructed to use the machine and perform the tests. Via VPN the virology technicians performed quality assurance of the results. Validated results were transferred to the LIS for documentation. ED doctors were instructed to use rapid PCR for all patients presenting with influenza-like symptoms when hospital admission was considered.

## **RESULTS**

A total of 67 tests were performed during 2015/2016 influenza season and 11 positive cases were detected. Samples of patients with negative result were also sent to the department of virology and in seven cases other respiratory viruses than influenza were detected. During the same period the virology laboratory received 150 samples from the ED which had not been tested by rapid PCR and in 18 cases influenza virus was detected. Clinically, 80% of influenza positive cases had at least three of the typical influenza-like symptoms whereas 79% of the negative cases had less than three symptoms. Fever was present in all but one influenza patients. Virology technicians were called three times to help with technical problems.

## **CONCLUSIONS**

Rapid influenza PCR helps to improve patient management in the ED. Deciding which patient should be tested by rapid PCR seems to be a bigger problem than the technical use of the test. To avoid high costs training of ED staff is essential

036

### MOLECULAR EPIDEMIOLOGY AND DISEASE SEVERITY OF RSV INFECTION IN CHILDREN FROM DIFFERENT GEOGRAPHICAL AREAS IN SUB-SAHARAN AFRICA

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#### BACKGROUND-AIM

Respiratory syncytial virus (RSV) is a major pathogen of acute respiratory tract infection (ARTI) and is the leading cause of hospitalization with severe bronchiolitis and pneumonia among infants and young children. In resource-poor settings, RSV testing is not routinely performed, and little is known about the molecular epidemiology of RSV in Sub-Saharan Africa. This research project investigates the interrelation between molecular epidemiology and clinical features of RSV infection amongst children in Mbeya/Tanzania, Nouna/Burkina Faso, Lambaréné/Gabon and Agogo/Ghana.

#### METHODS

Pharyngeal swabs were analysed from febrile children who presented with clinical symptoms of ARTI at one of the study sites (Ghana: February 2014-April 2015, n=484; Gabon: August 2015-March 2016, n=196; Tanzania: June 2015-June 2016, n=226; Burkina Faso: October 2015-March 2017, n=229). Disease severity was estimated using a standardized score derived from corresponding clinical data. Samples were analysed for RSV, influenza and other respiratory viruses using real-time PCR. For RSV positive samples, sequence analysis of the second hyper-variable region was performed to identify the emergence and global spread of circulating genotypes of RSV strains.

#### RESULTS

In total, 47 samples were tested RSV positive (Ghana n=30 (6.2%), Gabon n=4 (2%), Tanzania n=9 (4.0%), Burkina Faso n=2 (0.9%). Phylogenetic analysis revealed that the majority of RSV-A strains cluster with strains of the novel ON1 genotype with a 72-nucleotide duplication first described in Ontario, Canada in 2011. The ON1 genotype has replaced most of the previously described RSV A genotypes and now starts to further subdivide into ON1 sub-genotypes. Most RSV-B strains belonged to genotype BAIX.

#### CONCLUSIONS

This is the first phylogenetic data for RSV from Tanzania, Ghana, Burkina Faso and Gabon and will help to rank the epidemiological importance of RSV among other viral and bacterial causes of respiratory illness. Patterns of circulating RSV genotypes provide valuable information about the geographical spread, the transmission and the pathogenicity of RSV strains. Further surveillance of circulating genotypes in combination with corresponding clinical data is needed to understand their full implications.

037

### DETECTION OF RESPIRATORY VIRUSES: COMPARISON OF RT-PCR AND FILMARRAY (IDAHO TECHNOLOGY)

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#### BACKGROUND-AIM

Diagnostic of respiratory viruses is very important due to the epidemiological point of view and due to over-prescription of antibiotics. We compared the FilmArray RP (Idaho Technology, Inc., Salt Lake City, UT) and Real Time PCR which we used for routine diagnostic of respiratory viruses.

#### METHODS

From June 2012 to July 2016, 234 nasopharyngeal swabs (NP) were sent to the laboratory of the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, for the routine detection of respiratory viruses. Total nucleic acids were isolated from 190 µL vigorously vortexed NP swab medium on a MagNa Pure Compact instrument (Roche Applied science) according to the manufacturer's instructions. The NP swabs were initially tested with RT-PCR for respiratory syncytial virus (RSV), influenza viruses A and B (Flu A-B), parainfluenza viruses 1-3 (PIV 1-3), human metapneumovirus (hMPV), human coronaviruses (HCoV), human bocavirus (HBoV), adenovirus (AdV), human rhinovirus (HRV) and enteroviruses (EV). All samples were additionally tested for respiratory viruses by FilmArray Respiratory (RP) panel.

#### RESULTS

Viral etiology was confirmed in 135/234 (57.7%) patients by FilmArray RP and in 122/234 (52.1%) patients by RT-PCR that were sent to laboratory from June 2012 to July 2016. Among pathogens tested for by both methods, only results of RSV were identical between both methods. The greatest difference in agreement between methods was in detection of HRV and enteroviruses (EV). The FilmArray RP in the current configuration is also of limited diagnostic potential for diagnostics of small children with bronchiolitis, because HBoV was not included in respiratory panel.

#### CONCLUSIONS

FilmArray RP is easy to use and have short time to result. Therefore, and because of the high cost, the method is suitable only for urgent patients as point of care testing. Because of the increased number of samples during an outbreak, the method is only conditionally suitable for detection of respiratory viruses due to the limited scalability of the system and the high price.

038

### CLINICAL AND VIRAL EPIDEMIOLOGICAL CHARACTERISTICS OF ACUTE BRONCHIOLITIS IN TAIWANESE CHILDREN

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#### BACKGROUND-AIM

Acute bronchiolitis, one of the leading causes of hospitalization for young children, is caused by a diverse group of viruses. However, bronchiolitis actually is a heterogeneous condition. This study aimed to determine the frequency of viral pathogens causing acute bronchiolitis and to explore the association between different viral pathogens, clinical characters and laboratory findings in Taiwanese children.

#### METHODS

Nasopharyngeal aspirate specimens were collected from Chang Bing Show Chwan Memorial Hospital between October 2014 and December 2016. Viral nucleic acids were extracted by means of Quigen viral RNA/DNA extraction kit. The extract was tested for respiratory viruses using the respiratory virus panel xTAG RVP FAST v2, which simultaneously detects 19 different viruses and subtypes. Human rhinovirus(HRV) infection was confirmed by using HRV-specific polymerase chain reaction if the sample was positive for enterovirus/rhinovirus(RV-EV). The demographic data, clinical presentations, and laboratory findings of included children were obtained by medical chart review.

#### RESULTS

A total of 175 cases were enrolled throughout the study period. The mean age was  $14.4 \pm 9.1$  month-old, and the male accounted for 113 cases. At least one virus was detected in 175 (87.4%) of included children with single and multiple viruses in 67 and 33% of cases respectively. Respiratory syncytial virus (RSV) was the leading detected virus, accounting for 24.8% followed by rhinovirus/enterovirus in 24.2%, and RSV/HRV co-infection in 17.0% of cases. Two or more viruses (maximum, 5) were detected in 37.3% of included cases. HRV infection was confirmed in 94.6% of RV-EV positive specimens, which comprised HRV-A (35.7%), and HRV-C (41.4%) respectively. In comparison, the presence of fever, longer length of hospital stay, and lower white blood cell count were significantly associated with RSV infection ( $p < 0.05$ ). Instead, virus type or coinfection was not associated with disease severity.

#### CONCLUSIONS

In the present study, RSV and RV were the two leading viral pathogens for acute bronchiolitis. The clinical characters and laboratory findings were distinct from RSV and RV infection. No additional effect of dual RSV/RV infection was observed on the severity.

039

### RAPID DETECTION OF RESPIRATORY SYNCYTIAL VIRUS USING RT-SIBA®

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#### BACKGROUND-AIM

Rapid diagnosis of respiratory viruses improves patient management, minimizes unnecessary prescriptions of antibiotics, and prevents the spread of infections in environments which are prone to outbreaks, such as garrisons. Hence, we evaluated a rapid, portable molecular test system for near patient diagnostics with nasopharyngeal swab (NP) specimens obtained from conscripts with signs and symptoms of respiratory tract infection. We compared the performance of the novel, isothermal Reverse Transcription Strand Invasion Based Amplification (RT-SIBA®) assay with the conventional Reverse Transcription Real-Time PCR (RT-PCR) assay for detection of respiratory syncytial virus (RSV).

#### METHODS

A total of 120 coded NP specimens (60 RSV positives and 60 influenza positives) obtained from Finnish conscripts during the years 2011-2013 were retrospectively and anonymously analysed with the RT-SIBA RSV assay (Orion Diagnostica) and the RealStar® RSV RT-PCR assay (Altona Diagnostics).

The RT-SIBA assay targeted the nucleoprotein region of RSV, utilizing the recombinase-dependent insertion of a specific invasion oligonucleotide and primers after reverse transcription. The RT-SIBA reactions were run in the portable, fluorometric Orion GenRead® instrument for 35 minutes at a constant temperature of 41°C. The results were reported as positive, negative, or invalid by the automated software. The positive reactions were reported as soon as the reaction reached the level of positivity. In case of an invalid result, a new analysis was conducted.

#### RESULTS

The sensitivity and specificity of the RT-SIBA RSV assay, relative to the RealStar® RSV RT-PCR assay, were 90.2% (CI95 80.2–95.4) and 100% (CI95 93.8–100), respectively. The RT-SIBA RSV results were available in less than 20 min after starting the analysis run in the Orion GenRead® instrument. The sample preparation took 7 min per sample, including 2 min hands-on time and 5 min incubation time at 95°C.

#### CONCLUSIONS

The RT-SIBA RSV assay was found to be specific, sensitive, and easy to use. The assay combined a simple and effective sample preparation with ready-to-use, freeze-dried reagents with a stand-alone instrument. Therefore, it can be applied in decentralized settings, contributing to faster near-patient diagnostics.

040

# A ONE YEAR STUDY OF EPIDEMIOLOGY AND CLINICAL FEATURES OF HUMAN BOCAVIRUS ACUTE RESPIRATORY INFECTIONS IN SOUTH WESTERN FRANCE

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## BACKGROUND-AIM

Human bocavirus (HBoV) belongs to the family Parvoviridae and was first identified in 2005. HBoV has a worldwide distribution, its transmission and infection occur throughout the year with a predominance during winter and spring seasons. The role of HBoV in respiratory and/or gastroenteric infections remains unresolved even if it can be isolated from respiratory and digestive specimens during acute infections.

## METHODS

We retrospectively analyzed the clinical, epidemiological features from patients HBoV positive in respiratory samples from august 2015 to august 2016. Data were recovered from laboratory and hospitalization information systems. All respiratory samples (nasopharyngeal aspirates or bronchoalveolar fluids) had been tested with a real time multiplex PCR (Anyplex RV16 Seegene).

## RESULTS

A total of 3,926 patients were tested for respiratory viruses and, 1790 (45.6%) were positive for at least one virus; a multiple infection was found for 398 (10.1%) patients.

149 (3.8% of patients) respiratory specimen were positive for HBoV. It was alone for 37 (2.1%) patients while it coexisted with other viruses for 112 (6.2%) patients. HBoV was more common in viral coinfections than other viruses ( $p < 0.0001$ ). Rhino/enterovirus ( $n=68$ ) and Adenoviruses ( $n=40$ ) were the most common pathogens in coinfections.

The incidence of HBoV was higher ( $p=0.04$ ) during spring than during other season.

Sex ratio of positive patients was 0.52; median age was 1.14 years whereas mean age was 4.4 years [range 16 days – 90.1 years].

At admission 73 (49%) patients suffered from respiratory symptoms and 6 (4%) from gastroenteric symptoms. 15 (10.1%) patients were febrile.

57 patients had a past history of respiratory infections, 1 suffered from cystic fibrosis. 4 patients were bone marrow recipients.

7 (4.7%) patients declared a nosocomial infection.

The mean length of hospital stay was 9.3 days [0 – 124], all patients recovered.

## CONCLUSIONS

Human Bocavirus mainly targeted children aged 0-4 year old particularly during the spring season. The high prevalence of coinfections reinforces the question about the pathogenic roles of HBoV. Further studies including the determination of the viral genotyping should be carried out in order to appreciate the true pathogenicity of this agent.

041

# CLINICAL PERFORMANCE OF ALERE I RSV AND INFLUENZA A&B MOLECULAR TESTS FOR RAPID DETECTION OF RESPIRATORY SYNCYTIAL VIRUS AND INFLUENZA VIRUSES A AND B

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## BACKGROUND-AIM

This study evaluated the AlereTM i RSV and AlereTM i Influenza A&B assay for detection of Respiratory Syncytial Virus (RSV) or Influenza Viruses (IFV) A&B in respiratory specimens in comparison with real-time PCR. Both AlereTM i assays are isothermal nucleic acid amplification tests, which can deliver a result within 15 minutes with limited hands-on time. The assays are validated for nasal or nasopharyngeal swabs in viral transport media. In this study, not only swabs were analyzed, but also nasopharyngeal aspirates (NPA), bronchoalveolar lavage (BAL) fluid and sputum samples.

## METHODS

A selection of 32 (29 NPA, 3 swabs) and 45 (37 NPA, 4 swabs, 1 BAL and 3 sputa) clinical samples were analyzed with the AlereTM i influenza A&B and AlereTM i RSV, respectively. Real-time PCR (FTD FLU/HRSV, Fast Track Diagnostics) was used as reference standard.

## RESULTS

FTD FLU/HRSV RT-PCR detected IFVA in 20 out of 32 samples and IFVB in 8 of these specimens. The AlereTM i Influenza A&B correctly identified 27 of the IFV positives, missing one IFVA positive NPA with a RT-PCR CT-value of 28.48. As a result, the AlereTM i influenza A&B has a sensitivity of 96.4%. RSV was detected in 39 out of 45 specimens with the FTD FLU/HRSV RT-PCR. Thirty-seven of these were also detected by the AlereTM i RSV test, resulting in a sensitivity of 94.9%. The 2 RSV false-negative samples (NPA, swab) had high RT-PCR CT-values of 29.15 and 34.05. No false positives were obtained with the AlereTM i assays, resulting in a specificity of 100% for both assays.

## CONCLUSIONS

The AlereTM i influenza A&B and RSV assays are excellent point of care test for detection of RSV and IFVA&B as they generate a result quickly with limited hands-on time, are very easy to perform and have high sensitivity and specificity compared to RT-PCR.

042

# COMPARISON OF THE NEW CORIS INFLUA+B K-SET AND BD VERITOR FLU A+B FOR RAPID DETECTION OF INFLUENZA VIRUSES IN RESPIRATORY SAMPLES FROM THREE CONSECUTIVE FLU SEASONS IN BELGIUM

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## BACKGROUND-AIM

This study evaluated the clinical performance and user friendliness of a new commercial rapid antigen assay, the Influa+B K-Set (Coris BioConcept) in comparison with the established VeritorTM Flu A+B (Becton Dickinson) for the detection of influenza viruses (IFV) in nasopharyngeal specimens. A commercially available real-time PCR (FTD FLU/HRSV, Fast Track Diagnostics) was used as reference standard.

## METHODS

Nasopharyngeal aspirates (n=198) from 192 patients (169 children, 23 adults) were included in the study. Samples were collected during 3 consecutive flu seasons (2014-2017) in a Belgian tertiary hospital and were kept frozen or refrigerated until testing. All samples (78 fresh, 120 frozen) were analyzed using Influa+B K-Set, VeritorTM Flu A+B and FTD FLU/HRSV RT-PCR.

## RESULTS

In the 198 nasopharyngeal aspirates, 53 (27%) were found positive for IFVA and 9 (5%) for IFVB by FTD FLU/HRSV RT-PCR. Of these positives samples, the Influa+B K-Set detected 43 IFVA and 5 IFVB (3 invalid results) while the VeritorTM Flu A+B detected 47 IFVA and 7 IFVB (1 invalid result), resulting in sensitivities of 81.4 and 88.5% for Influa+B K-Set and VeritorTM Flu A+B respectively. The Influa+B K-Set false negatives had RT-PCR CT-values ranging from 22.66 to 28.92 while the CT-values of the VeritorTM Flu A+B ranged from 25.89 to 28.92. Influa+B K-Set generated 2 false positive results and VeritorTM Flu A+B 3, resulting in specificities of 98.5 and 97.8% respectively. The overall processing time of the Influa+B K-Set and VeritorTM Flu A+B is 17 and 12 minutes per specimen respectively, with a hands-on time for both around 2 minutes. The Influa+B K-Set requires visual interpretation which can be challenging especially when test lines are very faint. The VeritorTM Flu A+B test, on the other hand, can be analyzed using a digital reader, which eliminates this subjectivity.

## CONCLUSIONS

Both rapid antigen tests performed well in detecting IFV in nasopharyngeal aspirates, with a higher sensitivity for the VeritorTM Flu A+B test. Visual result interpretation of the Influa+B K-Set requires trained lab technicians, while the digital reader of the VeritorTM system minimizes operator errors.

043

# CLINICAL PERFORMANCE OF THE IDYLLA RESPIRATORY PANEL FOR MOLECULAR DETECTION OF INFLUENZA A/B IN PATIENTS PRESENTING TO PRIMARY CARE WITH INFLUENZA-LIKE ILLNESS

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## BACKGROUND-AIM

Influenza virus (IFV) is a highly contagious virus, causing acute respiratory illness and is often encountered in primary care. A point of care test (POCT) for IFV would make it possible for general practitioners (GPs) to distinguish true from other causes of influenza-like illness (ILI) and better target advice and treatment. We set out to determine the performance of the IdyllaTM Respiratory Panel (Idylla) for diagnosing respiratory infections caused by IFV using a commercially available real-time PCR test as reference standard (Fast Track Diagnostics Respiratory pathogens 21 plus assay (FTD)).

## METHODS

Patients with symptoms of ILI were enrolled in a prospective study as part of the PREPARE project ([www.prepare-europe.eu](http://www.prepare-europe.eu)) in European GP-practices during the past two seasonal influenza epidemics. Respiratory samples were obtained using nasal and oropharyngeal flocked swabs for paediatric patients and nasopharyngeal flocked swabs for adults. A subset of samples (n = 195) were analysed using Idylla fresh as well as after a freeze-thaw cycle to assess its use as an off-site analyser. A total of 651 frozen samples were analysed by Idylla and FTD.

## RESULTS

In samples analysed fresh, Idylla detected 79 (41%) IFVA and 35 (18%) IFVB. A freeze-thaw cycle resulted in a loss of 3 IFVB positives with low viral load and a gain of 2 IFVA positives. The analysis of the frozen samples resulted in a positivity rate of 34% IFVA and 17% IFVB. With FTD as reference standard, Idylla had a sensitivity of 98.6% for IFVA and 93.9% for IFVB, and a specificity of 98% for IFVA and 100% for IFVB. Other pathogens detected by FTD were *Staphylococcus aureus* (16%), *Streptococcus pneumoniae* (13%), coronavirus (14%), rhinovirus (9%), human metapneumovirus (5%), bocavirus (3%), RSV (2%), adenovirus (2%), *Mycoplasma pneumoniae* (1%) and enterovirus (1%).

## CONCLUSIONS

Idylla is a promising POCT for the detection of IFV in patients presenting to primary care with ILI. It requires minimal training and hands-on time to get test results in less than an hour with excellent clinical performance using a commercially available real-time PCR method as reference standard. In addition, Idylla produces similar results in fresh and frozen samples.

044

# EVALUATION OF A MULTIPLEX ASSAY IN THE CHARACTERIZATION OF LOWER AIRWAY TRACT INFECTIONS IN COPD PATIENTS

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## BACKGROUND-AIM

Subjects with moderate Chronic Obstructive Pulmonary Disease (COPD) may be frequently affected by bronchial exacerbations with many previously treated events (GOLD 2017). For this reason, in patients of the Thoracic Endoscopy Service who had a recent bronchial exacerbation were collected alveolar bronchial fluid specimens. In this study we combined a multiplex PCR assay for detection of respiratory viruses and pathogens with cultural assay to common germs. This study aims to investigate the value of the molecular approach in the diagnosis of recurrent acute bronchopneumopathy.

## METHODS

131 BAL were collected at Sacro Cuore Hospital, Negrar, between April and December 2016. The samples were from adult patients affected by COPD, asthma, bronchiectasis and recurrent exacerbations undergoing bronchoscopy. At collection the samples were split into two aliquots and processed in parallel by bacterial culture (routine method) and by molecular testing using FTD® Respiratory pathogen 33 (Fast-track diagnostics Ltd, Malta) after DNA extraction with ELiTe STAR Extraction System (ELITechGroup S.p.A., Italy).

## RESULTS

Over 131 BAL samples, the positive cases were 54% (71/131) at PCR and 41% (54/131) at routine test. The culture-positive samples were only 5% (5/131) and contained pathogens not among those detectable by the molecular assay. In the molecular test, the positives for viruses were 16% (20/131), alone (50% of 10/131) or in combination with bacteria (50%), with prevalence of Adenovirus, Coronavirus and RSV. The positives for bacteria only were 47% (61/131) of cases, with prevalence of Staphylococcus Aureus, Streptococcus Pneumoniae and Haemophilus Influenzae. Microorganism difficult to be isolated by culture, as Pneumocystis carinii and Bordetella pertussis, were also detected by PCR.

## CONCLUSIONS

Multiplex assays targeting a broad range of respiratory pathogen provide a valuable tool for:

- characterization of the low airways microbiome, including viruses
- detection of coinfection virus- bacteria
- monitoring infections during the antibiotic therapy

045

# GENOTYPING OF HUMAN RHINOVIRUSES IN CHILDREN WITH ACUTE RESPIRATORY TRACT INFECTION IN TURKEY, ISTANBUL

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## BACKGROUND-AIM

Respiratory viruses play significant role in acute respiratory tract infections (ARTI). Human rhinovirus (HRV) is considered to be one of the most frequent causes of ARTI. More than 150 HRV genotypes have been described and divided into three main groups, (A, B, and C). In this study, we analysed HRV genotypes in ARTI in pediatric patients group.

## METHODS

In this study randomly selected 39 samples were included, which were obtained from children with ARTI during 2015-2017. The noncoding region of HRV genome was amplified by semi-nested RT-PCR and the amplicons were sequenced bidirectionally. Phylogenetic tree constructed by MEGA 7.

## RESULTS

HRV detection rates according to years were 20% in 2015, 21,9% in 2016 and 28,63% in 2017. The RV-A genotype was detected in 46,2%, RV-B genotype in 12,8% and RV-C genotype in 41,0% of the patients. The distribution of HRV genotypes according to the clinical presentations of HRV infections were as follow: HRV-A in 41,2%, HRV-B in 17,6%, and HRV-C in 41,2% of upper respiratory tract infections and HRV-A in 50,0%, HRV-B in 9,1%, and HRV-C in 40,9% in lower tract respiratory infections. The distributions of HRV genotypes were not significantly different based on age and gender of the subjects.

## CONCLUSIONS

Viral etiological agents that cause severe ARTI pose a serious public health problem globally. In addition to new emerging pathogens like MERS, reemergence of viral pathogens with a increased virulence necessitates close monitoring of these kind of agents.

046

**VALIDATION OF THE VIRAL PANELS OF ALLPLEX™ RESPIRATORY ASSAY**I. Melara De La Cruz<sup>1</sup>, M. Kjellin<sup>1</sup>, A. Hyder<sup>1</sup>, S. Sharif<sup>1</sup>, K. Bondeson<sup>1</sup>, C. Öhrmalm<sup>1</sup><sup>1</sup>Dept of Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden**BACKGROUND-AIM**

The aim was to compare the virus panels of Allplex™ Respiratory Panel Assays (Allplex, Seegene) with Anyplex II RV16 (Anyplex, Seegene) and two in house RT-PCR for Influenza A/H1N1 and InflB/RSV (MB).

**METHODS**

- Allplex: 16 viruses and three influenza A virus subtypes. Panel 1: Influenza A (H3, H1, and H1pdm09), respiratory syncytial virus A (RSV A), RSV B, and Influenza B. Panel 2: adenovirus, parainfluenza 1, 2, 3, 4, and enterovirus. Panel 3: coronavirus OC43, NL63, and 228E, rhinovirus and bocavirus. It's a one-step PCR of 2 h 20 min with Ct-value.

- Anyplex: the same 16 viruses as Allplex (no Infl A typing). Two-step PCR (70 min RT, 3.5 h amplification and melting point analyses).

- MB: two different one-step in house PCR analyses, InflA/H1N1 and RSV/InflB RT-PCR, of 2 h 20 min.

Total nucleic acid extractions from year 2012, aliquoted and frozen at -70 °C, of respiratory tract samples positive for different viruses were analyzed with all three methods. Further, all respiratory tract samples during two weeks (week 5 and 15, 2016) were analyzed with Allplex and compared with Anyplex and/or MB.

In total, 119 samples were analyzed and compared between MB and Allplex panel 1, and 133-165 samples were analyzed and compared between Anyplex and Allplex panel 1, 2, 3.

**RESULTS**

Allplex showed a specificity of 99-100% for Infl A, Infl B and RSV, and a sensitivity of 78% (Infl B) and 100% (Infl A och RSV) when compared with MB.

Comparing Allplex with Anyplex demonstrated a specificity of 99-100% and a sensitivity of 81%-100% for Infl A, Infl B, RSV A and RSV B.

The other viruses had a specificity of 88%-100% (95% CI 54%-98%) and a sensitivity for AdV, PIV1, PIV2, MPV, BoV, RV and CoV of 81%-100% (95% CI 42%-71%). The sensitivity for EV, PIV3, PIV4, CoV NL63 och CoV 229E were 50-100%, but only 95% CI of 10-35% due to few positive samples.

Allplex detected Enterovirus D68, which Anyplex could not.

**CONCLUSIONS**

Conclusively, the specificity and sensitivity of Allplex were high compared with MB and Anyplex for all pathogens where we could analyze many samples. Allplex could detect Enterovirus D68. Further, Allplex has a shorter TAT than Anyplex, leading to an improvement for the patients who could receive answers of 16 pathogens the same day.

047

**FREQUENCY OF RESPIRATORY VIRUSES IN INFANTS WITH SEVERE RESPIRATORY INFECTION ADMITTED TO NEONATAL INTENSIVE CARE UNIT, MILAN (ITALY), 2015 AND 2016**L. Pellegrinelli<sup>1</sup>, S.C. Uceda Renteria<sup>2</sup>, L. Greco<sup>2</sup>, D. Guarneri<sup>2</sup>, V. Pizzo<sup>2</sup>, S. Binda<sup>1</sup>, E. Pariani<sup>1</sup>, G. Lunghi<sup>2</sup><sup>1</sup>Department of Biomedical Sciences for Health, University of Milan, Milan, Italy<sup>2</sup>Virology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Fondazione Cà Granda Ospedale Maggiore Policlinico, Milan, Italy**BACKGROUND-AIM**

Severe acute respiratory infections (SARI) are a leading cause of hospitalization and death in children. We report the frequencies of respiratory viruses molecularly detected in samples collected from infants with SARI admitted to the neonatal intensive care unit (NICU) of a University and research hospital in Milan (Italy) in 2015 and 2016.

**METHODS**

217 respiratory specimens collected from as many children aged less than 1 year (median age: 1.1 months; IQR: 2.1 months) who required NICU admission due to SARI were analysed. After nucleic acid extraction (EZ1@DSP Kit, Qiagen), samples were tested by a multiplex real-time PCR (Anyplex™ II, RV16-Detection, Seegene) to detect: Adenovirus (AdV), Bocavirus (BoV), Coronavirus (CoV), Parainfluenza virus (PIV), Metapneumovirus (MPV), Enterovirus (EV), Respiratory syncytial virus (RSV), Rhinovirus (RV), and Influenza virus (IV).

**RESULTS**

40.6% (88/217) of samples were positive for at least one of the studied viruses; more than one virus was detected in 9.1% (8/88) of these positive-specimens. A viral infection was recognized in 48.5% (48/99) of neonates (infants <1 month of age). RV, RSV and PIV were the 3 main contributors, detected in 88.6% (78/88) of positive-samples (48.9%, 28.4% and 11.4%, respectively). 17% (15/88) of positive-samples tested positive for EV, BoV or CoV (5.7% each). AdV, MPV and IV were identified in less than 6% of positive-samples (2.3%, 2.3% and 1.1%, respectively).

About half (41/77; 53.2%) of positive-samples was recorded from December to March, when 31.7% (13/41) of RV and 41.5% (17/41) of RSV infections occurred. All EVs were detected in the last 10 days of May 2015.

**CONCLUSIONS**

In our SARI series, at least one virus was identified in 40% of cases and nearly half occurred in neonates. The main contributor was RV, followed by RSV and PIV. In this study, the other viruses were low-frequency identified. Respiratory viruses were detected throughout the year, though more than half were recognized during winter months.

Routine molecular diagnosis of respiratory viruses in infants with SARI requiring NICU should be strongly encouraged to quickly identify the involved pathogens, to assess their spread and recognise outbreaks and to drive treatment, thus avoiding the useless administration of antibiotics in such young patients.

048

### EVALUATION OF LIFERIVER RESPIRATORY VIRAL KIT IN A HOSPITALIZED POPULATION

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### BACKGROUND-AIM

Virus is the main cause of respiratory infections. Our study compared six real-time RT PCR kits from Liferiver, CA, USA (Enterovirus (EV), Influenza virus A&B (IFV), Influenza A virus (H1N1), Influenza B typing (IBV), Respiratory Syncytial virus (RSV) typing A&B and Human Rhinovirus (HRV)) to our standard of care (SOC) process. Performances (sensitivity (Se), Specificity (Sp), Positive and Negative Predictive Values (PPV and NPV) and repeatability (coefficient of variation (CV)) were determined.

### METHODS

Assays (n=370) were retrospectively performed on 190 respiratory samples (broncho-alveolar lavage, nose-throat swabs, nasopharyngeal/tracheobronchial aspirates) collected in patients with respiratory tract infections, hospitalized in the Hospices Civils de Lyon, Lyon, France. All samples were characterized (viruses types and subtypes) at the National Reference Centers for Enteroviruses and Respiratory viruses, Lyon, France. Our referential consider concordance of  $\geq 2$  molecular testing specific of each viral target. Verification after a new extraction was performed for discordant samples with our SOC. The repeatability of each of the six tests was tested on ten replicates of three samples (low, median and high viral load, Ct).

### RESULTS

Performances could be obtained for each viral target after testing of 30 positives, 10 negatives samples for each viral target. Se, Sp, PPV and NPV were quantified at: i) 86.7%, 100%, 100%, 71.4% for EV kit; ii) 86.7%, 100%, 100%, 92.6% for IFA detection and 96.0%, 100%, 100%, 96.2% for IFV B detection using the IFV kit; iii) 50%, 100%, 100%, 40% for A(H1N1) pdm09 kit; iv) 100%, 100%, 100%, 100% for Yamagata lineage detection and 66.7%, 100%, 100%, 83.3% for Victoria lineage detection using the IBV kit; v) for RSV kit with RSV subtype A mix 100%, 48.0%, 53.6%, 100% and for RSV subtype B mix, 100%, 100%, 100%, 100%; vi) for HRV kit 90% 100%, 100%, 76.9%. Median CV has been estimated to 1.21% on a median Ct = 29.9 for EV; 0.58% on a median Ct= 23.1 for IFV; 1.48% on a median Ct= 30.8 for H1N1; 1.97% on a median Ct= 27.7 for IBV; 1.03% on a median Ct=18.6 for RSV; and 3.68% on a median Ct=20.8 for HRV.

### CONCLUSIONS

Performances were sufficient for most of the kits but isolated detection of H1N1 and dual mixes for detection of both RSV types remain perfectible.

049

### A NOVEL HUMAN METAPNEUMOVIRUS CARRYING A 111-NUCLEOTIDE DUPLICATION WITHIN THE G GENE DETECTED AT A TERTIARY UNIVERSITY HOSPITAL IN CATALONIA SINCE THE 2015-2016 SEASON

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### BACKGROUND-AIM

HMPV is an etiologic agent of upper and lower respiratory tract infection (RTI). It is an enveloped, negative-sense, single-stranded RNA virus belonging to the Pneumoviridae family. Based on coding sequences of the glycosylate (G) protein, which is one of the major envelope glycoproteins, HMPV is classified into two genotypes (A and B) and subdivided into 4 subgenotypes: A1, A2 (A2a and A2b lineages; A2b1 and A2b2 sublineages), B1 and B2.

### METHODS

Respiratory specimens from patients with suspicion of RTI attended or admitted to Hospital Universitari Vall d'Hebron (Barcelona) were received in the laboratory for respiratory virus confirmation from October 2015 to May 2017. All samples were analyzed by direct antigen detection immunofluorescence or real-time multiplex RT-PCR assays. Partial G coding sequences from HMPV viruses were sequenced to perform phylogenetic analyses and molecular characterizations in MEGA v5.2. Prediction of potential N- and O-glycosylation sites was performed with the NetNGlyc and NetOglyc servers, respectively.

### RESULTS

A total of 20,130 specimens from 14,768 patients were collected, of which 423 (2%) samples of 407 (3%) patients were HMPV laboratory-confirmed. Overall, phylogenetic analyses revealed that 11 (3%) samples belonged to A2a lineage, 192 (50%) to A2b, 105 (27%) to B1 subgenotype and 77 (20%) to B2, though the prevalence of a particular genotype changed season after season (2014-2015: B; 2015-2016: A, 2016-2017: A/B). Molecular characterization of 155 A2b2 sequences revealed that 13 viruses carried a 111-nucleotide duplication in the G gene, which recently increased its prevalence (from 2% of A2b2 viruses with the duplication during the 2015-2016 season to 16% in the last; p=0.004). With this duplication, the G protein further acquired 12-13 additional potential O-glycosylated sites.

### CONCLUSIONS

A 111-nucleotide duplication within HMPV G gene is described here, which is the second duplication event observed for this virus. The increase of the prevalence of this novel viral variant might suggest an evolutionary advantage related to this duplication. The acquisition of additional potential O-glycosylation sites might be associated with new antigenic features or changes in immune evasion mechanisms.

050

# DIAGNOSTIC PERFORMANCE OF RESPIRATORY SYNCYTIAL VIRUS RAPID ANTIGEN-BASED POINT OF CARE TEST DURING THE 2016/2017 FLU SEASON

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## BACKGROUND-AIM

Human Respiratory Syncytial Virus (RSV) is accountable for acute respiratory tract infections during winter months (November through April) especially in young infants and elderly people, being a leading cause of hospitalization. A rapid and sensitive diagnosis of RSV is important to provide optimal patient care, and to avoid inappropriate antibiotic use or nosocomial transmission. By comparing to molecular based diagnosis, performance of the rapid antigen detection test SOFIA RSV (Ingen) was evaluated in our hospital.

## METHODS

A retrospective study was carried out on 147 RSV-positive respiratory samples from 137 patients (104 infants, 33 adults) during the 2016/2017 flu season (December 31st through February 7st) in Rennes University Hospital (France). Routine diagnosis of RSV infection was based on RT-PCR, Anyplex RV 16 Detection (Seegene, n=73) or Xpert Flu/RSV XC (Cepheid, n=74). Samples stored at -80°C were retrospectively analyzed with SOFIA RSV test and qualitative SOFIA results were compared to those obtained by RT-PCR (Ct values) considered as gold standard.

## RESULTS

Of 147 RSV-positive respiratory samples by RT-PCR, 113 (77%) were SOFIA positive (median ct-value=19). When taking into account only the 113 positive respiratory samples from patients younger than 19, as recommended in manufacturer's instructions, sensitivity increased from 77% to 84% (95/113) with a median ct-value of 19 for the 95 SOFIA positive results. Eighteen results were considered false-negative (median ct-value=31, range=24 to 41) thus confirming the strong influence of viral load on SOFIA reactivity. A theoretical best sensitivity of 95% could be obtained for samples reaching a Ct lower or equal to 28 (91/95).

## CONCLUSIONS

The SOFIA RSV test showed good sensitivity when compared to the gold standard RT-PCR, especially for young people under 19 years. The short turn-around time (20 minutes) and the ease of use maybe helpful locally in pediatric wards to provide a fast result. The winter season 2016-2017 showed an unusual epidemic context in France with co-circulation of influenza virus and RSV. In such situation, a combined molecular detection of flu and RSV seems more suitable and cost-effective, particularly in the general population.

051

# GENOTYPES OF HUMAN RHINOVIRUSES IN CHILDREN AND ADULTS PATIENTS WITH ACUTE RESPIRATORY TRACT INFECTIONS

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## BACKGROUND-AIM

Human rhinoviruses (RV) cause a wide spectrum of disease, ranging from a mild influenza-like illness (ILI) to severe respiratory infection. Recent studies report that RV-A, B and C may be associated with both the common cold and severe lower respiratory infection. The main objective was to investigate of RV species by sequence analysis RV positive in nasopharyngeal samples among the patients with respiratory tract infections. Secondly, to establish the relationship between species detected and the age, the gender and the clinical diagnosis of the patients.

## METHODS

Nasopharyngeal swabs with RV detected from 127 who admitted to our hospital with acute respiratory symptoms were included between January 2014 and January 2016. Viral loads from 96 samples with 50 children and 46 adults were suitable for analyzing. The samples were tested for RV using real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Sequencing data of VP4/VP2 coding regions on RT-PCR-positive specimens were tested. Phylogenetic analysis was performed with Geneious 9.1.3 program. In the analysis, a 400 bp region of VP4/VP2 rhinovirus capsid protein coding region was included. Rhinovirus species were determined by analysing the sequences with references for VP4/VP2 coding region by neighbour-joining method.

## RESULTS

Out of total 95 samples, 65 (32 adult and 33 children) exhibited bands corresponding to the VP4 / VP2 gene regions using PCR. using PCR and were further subjected to sequencing. After sequence analysis, 28 were (43,07%) RV-A, 7 were (10,76%) RV-B and 28 were (43,07%) RV-C; moreover one sample was EV-D68 (1,53%) and one sample was EV-C (1,53%). The distribution of species in adults was: 15 (48,3%) RV-A, 5 (16,1%) RV-B and 11 (35,4%) RV-C. The distribution of species in children was 13 (%40,6) RV-A, 2(%6,3) RV-B and 17 (%53,1) RV-C. There was no statistically significant difference between RV species and clinical diagnosis, age and gender in both of the age groups (p> 0,05).

## CONCLUSIONS

In conclusion, the frequency of RV-A and RV-B species were found similar but higher than RV-B in whole study group. RV-A is more frequent in adults, while RV-C is more frequent among children. This was the first study from Turkey that reported the RV species.

052

# MOLECULAR EPIDEMIOLOGY AND CLINICAL PRESENTATION OF RESPIRATORY SYNCYTIAL VIRUS INFECTION AMONGST HOSPITALIZED CHILDREN IN ISLAMABAD/PAKISTAN

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## BACKGROUND-AIM

The aim of this research project is to understand the interrelation between molecular epidemiology and clinical features of respiratory syncytial virus (RSV) infection amongst hospitalized children (<5 years) in Islamabad/Pakistan.

## METHODS

We investigated RSV as well as influenza causing acute respiratory tract infection (ARTI), analysed patterns of circulating RSV subtypes/genotypes and determined their impact on the disease severity in Pakistan between February and April 2016. Nasopharyngeal swabs were obtained from hospitalized children (<5 years) who present with clinical symptoms of ARTI at the Paediatric Department of a Public Hospital in Islamabad/Pakistan. All nasopharyngeal swabs were analysed for RSV and Influenza using real time PCR. For RSV positive samples, sequence analysis of the second hyper-variable region of the RSV G gene was performed to obtain circulating sub-/genotypes.

## RESULTS

RSV-A was detected in n=10 (10.6%) and RSV-B in n=14 (14.9%) of all samples (n=94). Phylogenetic analysis revealed that the majority of RSV-A strains clustered with strains of the novel ON1 genotype with a 72-nucleotide duplication and all RSV-B strains belonged to genotype BAIX. The ON1 genotype is spreading worldwide becoming the predominant RSV-A strain but was not previously described in Pakistan.

## CONCLUSIONS

Mapping the spread of novel genotypes using data from different seasons and geographical areas can reveal transmission dynamics and the fitness of the viral strains. Further surveillance of circulating genotypes in combination with corresponding clinical data is needed to understand their full implications.

053

# DEVELOPMENT OF SYNTHETIC MULTIPLEXED QUALITATIVE EXTERNAL CONTROLS FOR MONITORING THE PERFORMANCE OF THE GENMARK DIAGNOSTICS EPLEX® RESPIRATORY PATHOGEN PANEL

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<sup>1</sup>GenMarkDx

<sup>2</sup>MMQCI

## BACKGROUND-AIM

Maine Molecular Quality Controls (MMQCI) Inc. has developed a unique, extractable multiplex control panel consisting of one negative control and four positive controls, designed to monitor all viral and bacterial organisms detected by GenMark's ePlex CE-IVD Respiratory Pathogen (RP) Panel (not currently for sale in the US).

## METHODS

The synthetic, multiplex molecular controls contain genome segments of all viral and bacterial pathogens detected by the RP Panel. The pathogens detected were designed in silico to create several single pieces of synthetic DNA, ligated into MMQCI vectors, and transformed to create stable frozen clone stocks. DNA plasmids and RNA transcripts were generated, quantified by 260/280 UV spec, and formulated in MMQCI's proprietary matrix.

## RESULTS

MMQCI's qualitative ePlex RP Control M306 demonstrated accurate results with 100% concordance for all 5 control tubes. Each Positive control tested (n=10), resulted in correct detection of relevant respiratory targets for all valid runs, and each Negative control (n=10) resulted in correct negative results. Clinical data consisted of 218 external controls tested across 6 sites. A total of 109 positive controls and 109 negative controls were tested and resulted in 97% concordance for all positive controls, and 95% concordance for all negative controls. Preliminary stability studies across two manufactured lots demonstrate real-time stability of 17 months, for the first lot, and 9 months, for the second lot, with accelerated stability indicating robust performance over 24 months when stored at -20 oC.

## CONCLUSIONS

MMQCI's synthetic, multiplex controls are designed to be part of an essential clinical laboratory quality control program. The multiplex nature of the controls streamlines manufacture, thus making them affordable for clinical labs. MMQCI's proprietary matrix and stabilization buffers allow for stable, reliable controls that are processed the same as a clinical sample to accurately simulate all pathogens detected by GenMark's ePlex CE-IVD Respiratory Pathogen Panel. Controls performed robustly at all sites with 99% accurate positive detection for all targets. The ePlex RP Control M306 is a ready-to-use, non-infectious, and well-characterized quality control panel that is highly effective for use in the clinical laboratory.

054

# **EVALUATION OF AN AUTOMATED PLATFORM FOR THE DETECTION OF RESPIRATORY SYNCYTIAL VIRUS AND INFLUENZA VIRUS IN COMPARISON WITH A MULTIPLEX REAL-TIME PCR ASSAY**

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## **BACKGROUND-AIM**

In winter, respiratory syncytial virus (RSV) and influenza virus (Flu) are considered the most common cause of respiratory infections among infants and young children (RSV) and adults and children (Flu).

The aim of this study was to evaluate the clinical performance of the Xpert®Xpress Flu/RSV test in comparison with Seegene Seeplex RV17® detection assay.

## **METHODS**

From December to February 2017, a total of 240 samples from patients admitted to the maggiore Ca' Granda Hospital (Milan) with respiratory symptoms, were collected and tested for Flu and RSV at the Virology Unit with Xpert®Xpress Flu/RSV test (Cepheid, Sunnyvale, CA USA) and Seegene Seeplex RV17® assay (Seegene, Inc., Seoul, South Korea).

## **RESULTS**

Among 240 specimens considered for comparison: 133 (55,4%) gave a positive result for at least one of the two virus tested on at least one of the platforms/tests evaluated; 81 (33,8%) samples gave a negative result for both. 26 (10,8%) samples had discrepant results. Concordance Index was 89,2%; K-Choen Index was 0,773 (0.61-0.80 = good concordance, according to guidelines).

Regarding Flu: 49 (20,4%) specimens were positive and 177(73,8%) negative. Concordance Index was 94,2%; K-Choen Index was 0,837 (0.81-1.00 = excellent concordance, according to guidelines).

Concerning RSV: 90 (37,5%) results were positive for RSV either for the Xpert®Xpress Flu/RSV test or for SeeGene Seeplex RV17; 136 (56,7%) results were negative for both. Concordance Index was 94,2%; K-Choen Index was 0,879 (0.81-1.00 = excellent concordance, according to guidelines).

## **CONCLUSIONS**

The Xpert®Xpress Flu/RSV is fully automated instrument with reduced and simplified handling, delivering negative results in 30 minutes and early detection of a positive sample in 20 minutes confirming it a valid choice for the screening of high-risk patients (immunocompromised, transplant patients etc). Data from this study demonstrated a high concordance between Xpert®Xpress Flu/RSV test and Seegene Seeplex RV17® assay. In addition, the majority of discrepant results were observed in samples with a ct value > 36. Among patients in therapy, the Xpert®Xpress Flu/RSV test was effective for post-therapy monitoring, a feature that should be expanded in future studies.

055

# **EVALUATION OF THE FUJI DRI-CHEM IMMUNO AG FLUAB KIT AND RSV/ADV KIT ON CLINICAL SAMPLES BY USE OF IMMUNO AG1 ANALYSER**

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## **BACKGROUND-AIM**

In France, the surveillance of acute respiratory infection in the general population is based on clinical samples performed by general practitioners and paediatricians from the Réseau Unique. Although PCR is becoming a method of choice for the diagnosis of respiratory viral infections, easy to handle rapid diagnostic tests are used in laboratories, as well as point of care. Here we evaluated the FUJI DRI-CHEM IMMUNO AG1 (FUJIFILM, Tokyo, Japan) silver amplification immunochromatography assay, for the detection of Influenza A&B and RSV. This system uses photographic development technology to increase the sensitivity of the conventional immunochromatographic assay.

## **METHODS**

This retrospective study compared the performance of FUJI DRI-CHEM IMMUNO AG FluAB kit with in-house RT-PCR for Influenza A&B detection and RSV/Adeno kit with commercialized RT-PCR (MWS r-gene™, BioMérieux, Lyon, France) for RSV detection. The clinical samples were tested for respiratory viruses by RT-PCR and subsequently stored at -80°C. The specimens selected for this evaluation were 340 nasopharyngeal swabs collected during two successive winter surveillance (2014/15 and 2015/16), including 100 positives for each influenza A, B and RSV viruses and 40 negatives.

## **RESULTS**

The IMMUNO AG allowed the detection of 46/100 Influenza A, 64/100 Influenza B and 67/100 RSV. All negative samples were negative and no false positives were detected. The threshold value for IMMUNO AG was determined by the Ct value of each RT-PCR. The threshold values were Ct=25 for Influenza A (Ct: 14.45 -28.8), Ct=21 for Influenza B (Ct: 14.1 -23.82) and Ct=19 for RSV (Ct: 15.2 -27.1).

## **CONCLUSIONS**

FUJI DRI-CHEM IMMUNO AG1 is a reliable system with a specificity of 100% for the detection of both Influenza A&B and RSV, the major virus pathogens responsible for outbreaks. Based on the threshold Ct values determined in this study, its sensitivity is high enough for rapid diagnostic testing. The ease of use of this system is of major of interest especially in the management of the patients during outbreaks. But these preliminary retrospective results need to be completed with prospective data.

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# **VALIDATION OF NUCLEIC ACID EXTRACTION METHODS ON A QIASYMPHONY FOR PCR TESTING OF MULTIPLE POST-MORTEM TISSUE TYPES**

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## **BACKGROUND-AIM**

The ability to extract nucleic acids from many different tissue types is important for post-mortem investigations. Different PCR assays are used depending on the deceased patient's history and tissue type. The most common targets are: CMV, EBV, VZV, enterovirus, adenoviruses, PVB19, HSV1/2, influenza A/B, RSV A/B and PIV1-4. The aim of this project was to validate a tissue nucleic acid extraction method compatible with our current laboratory procedures to allow streamlined in-house PCR testing.

## **METHODS**

From the 300 post mortem tissue samples that were received between 2013 and 2016, 7 samples known to be positive for one or more pathogens and 16 PCR negative samples were selected for this study. Samples were lysed according to modified protocol recommended by manufacturer. The extractions were performed on the Qiasymphony with either the recommended High Tissue Content protocol (no internal control), or the Complex 200 (C200) protocol, with MS2 as the internal control (IC). Testing was then performed by in-house RT PCR on the Rotor-Gene-Q for the RNP gene and MS2 for C200 extracted samples. The extraction protocol was tested with a mix of 4 internal controls, including MS2, T4, BMV and the Qiagen IC, where eluates were tested by RT PCR for the RNP gene, MS2, HSV/VZV, respiratory panel and CMV/EBV. Validation of the C200 extraction with the 4 internal controls was performed on 55 PCR positive and 24 PCR negative post mortem tissue samples.

## **RESULTS**

The DNA concentration of the eluates were comparable from the two extraction protocols (Paired T-test  $p=0.384$ ). CT values for the RNP PCR and for the viral target PCRs were also comparable for samples processed by each of the two extraction protocols. The performance of the Enterovirus PCR was more accurate with the C200 protocol with the 4 internal controls.

## **CONCLUSIONS**

This study demonstrated that the C200 protocol on the Qiasymphony performed as efficiently as the HTC protocol on multiple tissue types and was therefore chosen as the method for further validation. The MS2 and RNP PCRs were successfully used as extraction and run controls.

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# **RETROSPECTIVELY EVALUATION OF VIRAL RESPIRATORY TRACT INFECTIONS IN A UNIVERSITY HOSPITAL IN ANKARA, TURKEY**

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## **BACKGROUND-AIM**

Viruses are the agents of two third of acute respiratory tract diseases. These viral agents cause severe respiratory tract infections especially in children, elderly and immunosuppressed patients. The aim of this study is to detect respiratory tract viruses retrospectively in patients attended to our hospital.

## **METHODS**

Totally 310 patients that are composed of 136 female and 174 male, attended to Gazi University Hospital between 0-93 years old, from February 2016 to March 2017 were included in the study. Nucleic acid extraction was done by EZ1 Virus Mini Kit in EZ1 extraction device (Qiagen, Germany) from nasopharyngeal swab, throat swab or bronchoalveolar lavage (BAL) samples sent to our laboratory. Extracted nucleic acids were amplified by multiplex real time PCR method which can detect 19 viral pathogens (FTD Respiratory Pathogens 21, Malta) in Rotor Gene (Qiagen, Germany) device.

## **RESULTS**

Totally 319 samples from 310 patients with respiratory tract symptoms were sent from several clinics. Among these samples, 155 (48.6%) were positive for any respiratory viruses. The highest positivities were on February (68.7%) and March (64.1%), the lowest positivity was on July (17.6%). The most detected virus as a single agent was rhinovirus with the rate of 23.9% (37/155) among all positive cases and it was followed by influenza B virus with the rate of 11% (17/155). RSV A/B, HAdV, CoV NL63, CoV 229E, HPIV3, Influenza A, HMPV A/B, HBoV, CoV HKU1, CoV OC43, HPIV1, HPIV2 and HPeV was detected as 8.5%, 7.8%, 4.6%, 3.2%, 3.2%, 2.6%, 2.6%, 2%, 2%, 0.7%, 0.7%, 0.7% respectively. Among all positive patients, 23.9% (37/155) had multiple infections. The highest positivity rate was from paediatric infections clinic with 70% (39/56), followed by general paediatric clinic with 42.8% (12/28) and neonatal intensive care unit with 38.5% (10/26).

## **CONCLUSIONS**

Detection of respiratory viruses is helpful for unnecessary antibiotic use and vital for immunosuppressed and intensive care unit patients, especially neonates. Therefore determination of viral respiratory agents is essential in routine diagnostics. Additionally, it provides seasonal epidemiological data for viruses that circulate in region.

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# **VIRAL DETECTION OF VIRUSES FROM SPECIMENS COLLECTED WITH MSWAB™ USING DIRECT-RAPID NUCLEIC ACIDS EXTRACTION AND AMPLIFICATION AND CULTURE CONFIRMATION**

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## **BACKGROUND-AIM**

Molecular Rapid detection of viruses is important for patient care and can reduce spreading of infection. Extraction of nucleic acids can be costly and increases the results turnaround. MSwab™ is a molecular medium for the collection, and storage of clinical specimens for the detection of viruses with direct-rapid or traditional nucleic acid extraction and amplification assays and culture. It is compatible with in-house or commercial nucleic acids amplification assays and supports viral and bacterial viability for culture and antigens detection. Study's objectives were to validate MSwab™ for: 1) Direct-rapid and traditional nucleic acids extraction for the detection of viruses by real-time PCR. 2) Virus isolation by shell-vial culture.

## **METHODS**

Nasopharyngeal (N=80) and lesion swab (N=30) and saliva swabs (10) were used for this validation. Swabs from positive samples, first tested by real time PCR, including Flu A, Flu B, RSV, P1, P2, P3, Adeno, hMPV, HSV1, HSV2, and VZV were transferred in a tube of MSwab medium. Saliva swabs were collected from patients and placed in a tube of MSwab™ medium. Each MSwab™ tube was vortexed and 200 µl of sample were used to inoculate a shell vial culture, another 200 µl were added to a microtube and placed in a dry heating block at 1000C for 5min, vortexed for 10s and centrifuged at 14,000rpm for 2min. A 200ul aliquot was extracted with the easyMag™ (Biomérieux) and eluted in 55ul. Five ul of each nucleic acid extracted with both methods were tested with the in-house single or multiplex real time PCR and compared to the UTM results.

## **RESULTS**

MSwab direct-rapid and EasyMag nucleic acid extraction tested by real-time PCR and shell-vial culture confirmed all FA FB, RSV, P1, P2, P3, ADV, hMPV, HSV1, HSV2, VZV and CMV. No toxicity or contamination was observed in culture.

## **CONCLUSIONS**

The data obtained in this study demonstrated that the Copan MSwab™ can be used for direct-rapid nucleic acid extraction for the detection of viruses using single or multiplex real-time PCR and viral culture confirmation of new influenza strains or for antiviral resistance. MSwab™ direct-rapid extraction improves results turnaround time, save costly extraction reagents and supports culture confirmation.

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# **FIRST EVALUATION OF THE XPERT® XPRESS FLU/RSV FOR DETECTION OF INFLUENZA VIRUS**

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## **BACKGROUND-AIM**

Seasonal epidemics of influenza are responsible for significant morbidity and mortality worldwide.

Real-time RT-PCR assays for influenza are widely used. Rapid and sensitive detection has a significant advantage as it can optimize management by limiting administration of unnecessary antimicrobials and enhancing decision making on infection control practices

## **METHODS**

In this study, we evaluated the performance of the new Xpert® Xpress Flu/ RSV (Cepheid, Sunnyvale), a next generation automated, multiplex RT-PCR assay for the qualitative detection and differentiation of influenza A, influenza B and RSV.

The performance of the Xpert Xpress Flu/RSV assay for the detection of influenza was compared to the Alere Binax now Flu A&B and Alere i Flu A&B assays.

Fifty fresh consecutive samples were collected from ambulant and hospitalized patients from February 4th to February 13th 2017. They consisted of 41 nasopharyngeal swabs and 9 nasopharyngeal aspirates.

Testing and interpretation of results were done according to the assay package inserts.

Discrepant results were resolved by in house real-time PCR performed at the national reference lab (WIV-ISP) for influenza and other respiratory viruses.

## **RESULTS**

Of the 50 samples tested, 26 were positive for influenza A by Xpert Xpress Flu/RSV, 18 were positive for influenza A by Alere i and only 6 were positive for influenza A by Binax Now. The eight samples that were negative by Alere i and positive by Xpert were sent to the national reference lab WIV-ISP for analysis by in house real-time PCR. The reference lab confirmed 5 positive influenza A cases but argued that the other 3 discrepant samples were most likely positive containing an amount of virus below the detection limit of the in house PCR.

## **CONCLUSIONS**

Xpert Xpress Flu/RSV demonstrated better sensitivity (100%) versus the Binax now Flu A&B (23%) and Alere i Flu A&B assays (69%).

The accuracy and reliability associated with the 30 mins turnaround time makes the Xpert Xpress Flu/RSV suitable for point-of-care and routine diagnosis of influenza viral infections

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# CE-IVD VALIDATION OF THE MOLECULAR DIAGNOSTICS FLUA/FLUB/RSV ASSAY ON ELITE INGENIUS™ SAMPLE-TO-RESULT SOLUTION FOR THE RAPID DETECTION OF LOWER RESPIRATORY TRACT INFECTIONS

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## BACKGROUND-AIM

Influenza A (FluA), influenza B (FluB), and Respiratory Syncytial Virus (RSV) are common infectious agents responsible for seasonal respiratory infections. These viruses can be detected by molecular diagnostics methods from nasopharyngeal specimens. The introduction of a fully automated multiplex assay on a sample-to-result system drastically reduces hands-on time and simplifies laboratory workflow.

In the present study, the analytical verification and the clinical validation of the ELITechGroup Respiratory Viral ELITE MGB® Panel were carried out for the CE-IVD marking of this assay in association with ELITE InGenius™.

## METHODS

ELITechGroup Respiratory Viral ELITE MGB® Panel (ELITechGroup Molecular Diagnostics) is a triplex assay for the detection and the differentiation of FluA, FluB, and RSV. ELITE InGenius™ automatically performs nucleic acid extraction, amplification and results analysis integrating on a single platform with 12 extraction modules and 12 independently controlled thermocyclers.

The validation plan was based on (1) analytical studies with efficiency, linearity, sensitivity, repeatability and reproducibility testing to verify the PCR performance; (2) analytical specificity and system performance verification using certified reference material: QCMD INFRNA15 (Qnostics Ltd.).

The clinical study, including diagnostic sensitivity and specificity assessment, was performed by testing retrospective respiratory samples (n = 154).

## RESULTS

ELITechGroup Respiratory Viral ELITE MGB® Panel passed the validation criteria of analytical and clinical studies. Full concordance was observed on QCMD EQA panels.

Clinical sensitivity was calculated at 96% for FluA (24/25 positive samples), 100% for FluB (25/25 samples) and 100% for RSV (41/41 samples).

Clinical specificity was calculated at 100% (65/65 negative samples) for each target.

User intervention was limited to setting up the “walk away” analysis with hands on time of about 20 minutes.

## CONCLUSIONS

The excellent results obtained support the CE-IVD marking of ELITechGroup Respiratory Viral ELITE MGB® Panel in combination with the ELITE InGenius™ system for the detection of lower respiratory tract infections. The fully automated approach can improve laboratory workflow and resource management.

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# PERFORMANCE EVALUATION OF THE NEW FULLY AUTOMATED PANTHER FUSION RESPIRATORY ASSAYS FROM HOLOGIC, IN COMPARISON TO PROFLU+, PROFAST AND R-GENE RESPIRATORY ASSAYS

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## BACKGROUND-AIM

Fast and reliable detection of Influenza and RSV is important for infection control and antiviral treatment of Influenza in susceptible patients. Since Influenza like illness is caused by many different respiratory viruses, it is important to use highly specific laboratory tests.

The new Panther Fusion instrument system (Hologic Inc.) together with the Panther Fusion Respiratory assay panels, offers random access, which enables continuous loading of samples and resulting in short turnaround time, also in a laboratory with a high throughput.

## METHODS

In this study we compared the overall performance of the new assay panels Panther Fusion Flu A/B/RSV, Panther Fusion Paraflu 1-4 and Panther Fusion AdV/hMPV/RV, intended for use with the recently launched Panther Fusion system, with our routine tests; Hologic's ProFlu+ (FluA/FluB/RSV), ProFast (influenza typing) and bioMerieux's R-Gene (RSV/hMPV/RhV/EntV/ParaInf 1-4/CoV/AdV/BoV) kit. In a prospective study 400 patient specimens were tested side-by-side. In a retrospective part of the study 117 known positive samples were tested with the Panther Fusion kits.

## RESULTS

In the 400 samples in the prospective study, 207 and 195 samples tested positive for either virus on the Panther Fusion and the routine assays, respectively. Several discrepancies were found between the kits. Discordant testing is done through sequencing.

In the retrospective part of the study all samples tested positive with at least one of the three Panther Fusion Respiratory panels, with the exception of one sample, which was tested positive for Rhinovirus/Enterovirus with the bioMerieux R-gene kit. Furthermore, the Panther Fusion detected several viruses that had not been detected previously. Discrepancy analysis using sequencing in order to verify the presence of viral material is ongoing.

## CONCLUSIONS

In conclusion we find that the Panther Fusion along with the Panther Fusion Respiratory panels offer a fast and reliable way to detect respiratory viruses, although discrepancy analysis needs to be done. Furthermore, the random access capability of the Panther Fusion system significantly improves the turnaround time, which in turn allows more efficient infection control and earlier initiation of antiviral treatment.

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**COMPARISON OF TWO DIFFERENT MOLECULAR METHODS IN THE DETECTION OF RESPIRATORY VIRUSES**

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**BACKGROUND-AIM**

The clinical laboratory plays a key role in the diagnosis of respiratory tract infections however their complexity poses a challenge particularly for number of viruses involved and the ability of molecular methods to distinguish between real infections and colonization.

The object of our study is the comparison between two different multiplex real-time PCRs for the detection of viral infections during winter 2017

**METHODS**

Samples of nasopharyngeal swabs, aspirates and BAL were obtained from in-patients attending Ospedale S. Gerardo during January and February 2017.

Nucleic acid extraction of samples was performed with NucliSENS easyMAG (BioMerieux). Amplification was performed according to manufacturer indication with Allplex Respiratory Panel Assays (Seegene), a multiplex one-step real time RT-PCR, and Respiratory Viruses panel (NLM-Nuclear Laser Medicine), a multiplex nested real time RT-PCR. Potential targets detected with the two different kits are the same, except for Bocavirus that is detected only with Seegene

**RESULTS**

A total of 84 samples were analysed.

Seegene: 36 samples positive for 1 virus, 9 positive for 2 or more, 39 negative. Flu-A (H3) is the most frequent virus detected (15), then RSV-A (13) and RSV-B (11).

NLM: 25 sample positive for 1 virus, 24 positive for 2 or more, 35 negative. RSV-A (14) is the most frequent virus detected, then RSV-B and Flu-A (11). NL63 Coronavirus was detected in 29 samples but with a low copy number in 93%

Comparing raw results, 34 samples were completely concordant (40,5%), 40 samples were partially concordant (46,6%) and 10 were completely discordant (11,9%). After adjusting positivity threshold to eliminate uncertain data, concordant samples were 81% (68 samples), partial concordant were 9,5% (8), while completely discordant were 9,5% (8).

**CONCLUSIONS**

Both methods showed a good performance and TAT < 3 hours with all samples. Although methods are not true quantitative tests, most of the differences were found in samples with low viral load. Without a reference method to compare with, the low threshold of positivity of each methods can lead to identify a great number of positive samples and therefore an analysis of clinical meaning of low viral load samples is advisable in order to recognize true infected patients

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**EVALUATION OF THE DIAGNOSTIC PERFORMANCE OF A RAPID ANTIGEN TEST FOR ADENOVIRUSES, RESPIRATORY SYNCYTIAL VIRUSES AND INFLUENZA VIRUSES IN COMPARISON TO REAL TIME RT-PCR**

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**BACKGROUND-AIM**

Respiratory syncytial viruses (RSV), Influenza viruses and Adenoviruses belong to the most important viruses that can cause severe acute respiratory tract infections. A timely identification of these viruses is important, since it impacts disease management and isolation measures. In contrast to the gold standard – real-time RT-PCR – rapid antigen tests (RAT) are fast, cheap, and easy to use, which allow their use as a fast screening tool, especially in children.

**METHODS**

During the 2016-2017 respiratory season, 109 respiratory samples with a cycle threshold (Ct)-value ≤ 25 were selected to investigate the diagnostic performance of a chromatographic immunoassay from Biotec for RSV, Adenoviruses, and Influenza virus type A and B by comparing to a multiplex RT-PCR. To determine the threshold Ct-value for a positive result of the RAT, the samples were selected to give sufficient spread in Ct-values. The specificity was determined with samples from an array of viruses (Ct-values ≤ 16) that are similar to the tested viruses, or give comparable symptoms (Metapneumoviruses, Parainfluenza Viruses, and Coronaviruses).

**RESULTS**

If all Ct-values are included, the sensitivities lie between 50 and 69% for all viruses. If a threshold of Ct ≤ 20 is used, sensitivity remains 69% for RSV A, and shifts to 89% for RSV B. For Adenoviruses, Influenza A and B we find sensitivity values of 62%, 94% and 100%, respectively. The specificity of all tests is near 100%, with only one false positive for Influenza B.

**CONCLUSIONS**

The overall assay sensitivity is insufficient in the full Ct-range, which remains limited at the strongest Ct-values for RSV A and Adenoviruses. In part, this may be due to the study limitations: the low sample number, and the biased sample selection. Since Adenoviruses show a high natural variation, its detection is challenging, with low analytical sensitivities (22-67%) of RAT describes in literature. Its likeliness to a bacterial respiratory infection, however, stresses the relevance for its parallel detection, to which this RAT may contribute. Overall, a significant number of patients with severe symptoms could be missed. Still, the high specificity allows for an initial screening, but requires that negative results are confirmed with more advanced methods.

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# ANTIVIRAL ACTION OF HYDROMETHANOLIC EXTRACT OF GEOPROPOLIS FROM SCAPTOTRIGONA POSTICA AGAINST RUBELLA VIRUS REPLICATION

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## BACKGROUND-AIM

Researches on chemical composition and biological activity of propolis had been carried out mainly on Apidae specie *Apismellifera* L, which exhibited activity against some important virus. For the Meliponini species, there are few studies about their chemical composition and biological activities. This study evaluated the effect of geopropolis from *Scaptotrigona postica* on Rubella virus infected Rabbit Cornea cells.

## METHODS

Antiviral assay in cell culture, real-time PCR, and transmission electron microscopy were used to demonstrate that geopropolis inhibit the production of infectious Rubella virus. Viral binding, penetration assay was used to demonstrated the local of action and chemical analysis to define the structure was performed.

## RESULTS

Was not observed significant toxicity at concentration of 68ug/mL. Both posttreatment and pretreatment showed that geopropolis has antiviral activity with 17µg/mL which was sufficient to prevent viral penetration. These results were confirmed by qPCR. By TEM were not detected RV like particles into the cytoplasm when cells were treated with geopropolis and infected with RV. Phytochemical analysis of hydromethanolic extract of geopropolis using Reversed Phase technique was performed. The main compounds found were vicienin2; catechin arabinoside; catechin rhamnoside; schaftoside; 5-O-caffeoylquinic acid arabinoside and the alkaloid 7-(3-Methoxy-2-methylbutyryl)-9-echimidinyl retronectine. The MS/MS experiments in protonated hydroxycinnamic acid amide derivatives produced abundant fragment ions attributed to the acyl neutral losses, coumaric residue; caffeic residue and ferulic residue. Pirrolyzidine alkaloids were also found in this extract. The content of vicienin-2 and schaftoside in 13.8 mg/mL of geopropolis extract, were 0.022 and 0.009 mg/mL, respectively.

## CONCLUSIONS

Flavonoids, triacylated spermidines and other phenolic compounds can exhibit several important control functions in cells. This fact can explain the great potential of hydromethanolic extract of geopropolis from *Scaptotrigona postica* in the inhibition of RV, using a low concentration of extract, what could be attributed of the high content of flavones-C-glycosides. So the use of phytoterapics to treat viral infections could be explored.

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# COMPARISON OF RAPID ASSAY AND MOLECULAR ASSAY FOR DIAGNOSIS OF VIRAL AGENTS CAUSING RESPIRATORY TRACT INFECTIONS

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## BACKGROUND-AIM

Influenza virus and respiratory syncytial virus (RSV), which cause acute respiratory tract infections, are the main causes of hospital admission and workforce loss. Since they may require specific treatment (i.e. oseltamivir) and measures of infection control, it is important to identify these two viral agents for accurate management of the diseases.

The present study examines the prevalence of influenza A and B, and RSV among patients with symptoms of upper respiratory tract infection (URTI) from Sakarya Region.

## METHODS

The study included totally 24 patients (17 female, 7 male) who presented to Pediatrics and Infectious Diseases Clinics of Sakarya University Training and Research Hospital between January-April 2017 due to respiratory symptoms developing within the last 48 hours of presentation. The study was approved by the local ethics committee. Nasopharyngeal swab samples were transferred to microbiology laboratory and were analyzed with rapid chromatographic assay (Veritor System for Flu A+B, BD) for presence of Influenza A and B antigens. Confirmation of all test results were made with Influenza A/B, RSV multiplex RT PCR assay (BD, USA). Demographical and some clinical data were collected using a form.

## RESULTS

Mean patient age was 11 years (18 months-57 years). Regarding their symptoms, all patients had fever, 92% had nasal discharge, 67% had cough, and 25% had wheezing. Chromatographic assay results showed presence of Influenza A in 8 patients (33%), Influenza B in 5 patients (21%), and Influenza A+B in 2 patients (8%). All samples were analyzed with molecular assay, and the previous test results of the rapid assay for Influenza A and B were confirmed by molecular assay in all samples. In 1 sample (4%) that was negative for Influenza A or B, molecular test revealed presence of RSV.

## CONCLUSIONS

Identification of viral agents with rapid tests in patients presenting with URTI symptoms has great value for early detection of outbreaks, avoidance of unnecessary antibiotherapy, prompt initiation of accurate treatment, and prevention of hospital infections, and it reduces economical losses as well.

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### EV-D68 IN PEDIATRIC PATIENTS WITH A RESPIRATORY SYNDROME: THE CIRCULATION OF NEW B3 CLADE IN ITALY

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#### BACKGROUND-AIM

EV-D68 is an emerging infectious agent that has been found associated with both mild and severe respiratory diseases and neurological clinical manifestations. Four clades with a number of subclades that can circulate or co-circulate during different periods have been identified. However, molecular evolution of EV-D68 is not known as it is the possible association between specific genetic variants and the development of severe cases. To solve these problems, genetics of strains identified during an outbreak of EV-D68 infection that occurred in Italy during the period March-October 2016 were studied.

#### METHODS

Nasopharyngeal samples obtained from children admitted to the Emergency Room for respiratory infection were tested with a previously validated specific real-time PCR for detection and quantification of EV-D68. Phylogenetic analysis of the virus was performed sequencing the major capsid protein (VP1). Moreover, tests for detection of selective pressure were carried out.

#### RESULTS

Respiratory samples of 390 children were tested. Twenty-two patients (5.6%; median age, 47 months) were infected by EV-D68. All but three had a lower respiratory tract infection but none of these was severe, although in a few cases a transient reduction of SaO<sub>2</sub> level was evidenced at admission. All the strains belonged to the EV-D68 subclade B3. No evidence of increased clinical severity associated with specific molecular signatures of VP1 sequence or viral load was shown. B3 strains had 92.3% and 94.7% nucleotide identity with B3 clades identified in other countries where a number of very severe cases was diagnosed. No sign of selective pressure was found.

#### CONCLUSIONS

EV-D68 subclade B3 was the only cause of the EV-D68 diseases diagnosed in Italy during 2016. The same subclade was found in Northern Europe, China and the USA in the same period. This suggests that all the outbreaks had a common origin and EV-D68 B3 has become the preeminent EV-D68 strain causing disease worldwide. No specific characteristic of EV-D68 VP1 has been found associated with disease characteristics, in contrast with what has been evidenced with other studies. Further studies on full-genome sequencing in larger cohorts of patients are needed.

067

### INVESTIGATION OF CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF UPPER AND LOWER RESPIRATORY TRACT INFECTIONS AGENT RHINOVIRUS

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#### BACKGROUND-AIM

The viruses which can cause significant respiratory tract infections in certain age groups might create outbreaks and pandemics because of easy transmission. Investigation of the association among rhinovirus (RV) and age, season and underlying illnesses was aimed in this study.

#### METHODS

Totally 84 cases, attended to Gazi University Hospital Paediatric Infections, Paediatric Intensive Care and Neonatal Intensive Care Units, from March 2016 to March 2017 who has respiratory tract infection symptoms were examined retrospectively. Nucleic acid extraction was done by EZ1 Virus Mini Kit in EZ1 extraction device (Qiagen, Germany) from nasopharyngeal swab (UTM-RT transport, Copan Diagnostics, Italy) or throat swab samples sent to our laboratory. Extracted nucleic acids were amplified by multiplex real time PCR method which can detect 19 viral pathogens (FTD Respiratory Pathogens 21, Malta) in Rotor Gene (Qiagen, Germany) device.

#### RESULTS

Viral agents were detected in 49 samples (58%) among 84 samples. Rhinovirus was detected as a single agent in 13 (15.4%) patients and 10 (11.9%) patients had RV with other viruses. The highest RV and RV with other viruses positivity was detected in children under 1 year old (39%) and in March (17%) and November (17%). Rhinovirus positivity was mostly seen in spring and autumn. Thirty eight percent of RV positive patients had pneumonia and 30% of RV with other viruses positive patients had pneumonia.

#### CONCLUSIONS

Rhinovirus was the most frequent viral agent among 19 respiratory viruses in this study, similar to other studies. The studies about respiratory viruses indicate that RV positivity is higher in children younger than 1 year old. In our study, RV was also the most frequent agent in children younger than 1 year old. This result is significant for planning patient's follow up and regulating treatment. The seasonal evaluation of RV was also notable for spring and autumn. This finding might have been effected by geographical and humidity differences from other regions of Turkey. Additionally, considerable number of pneumonia and other respiratory diseases caused by viral agents is vital in immunosuppressive patients.

068

# **FACTORS AFFECTING COMPLIANCE WITH TUBERCULOSIS TREATMENT IN PAKISTANI PEOPLE**

A. Athar<sup>1</sup><sup>1</sup>Fatima Jinnah Medical University/Sir Ganga Ram Hospital Lahore

## **BACKGROUND-AIM**

Background: Tuberculosis is one of the major causes of disability and death worldwide. More than 95% of the deaths due to tuberculosis occur in low and middle income countries. Treatment defaulting is one of the major causes of failure of tuberculosis control programs in Pakistan.

Objectives: To evaluate association between various factors affecting compliance with tuberculosis treatment. Identification of factors can help improve compliance with tuberculosis treatment.

## **METHODS**

Method: A case control study was carried out to gain better understanding of impact of poverty, social stigmatization, and deficient health education, poor patient knowledge about the disease, pregnancy and treatment cost. 110 people with treatment failure, enrolled as cases were interviewed. Equal number of cured people enrolled as controls were interviewed. Cases and controls were enrolled from services hospital Lahore, Gulab devi hospital Lahore and Jinnah hospital Lahore.

## **RESULTS**

Results: Analysis revealed that majority of people with treatment failure were poor, they feared social stigmatization and they had deficient health education. Most of the female patients did not adhere to the treatment due to pregnancy.

## **CONCLUSIONS**

Conclusion: The findings of this study reveal the urgent need for health education to convince general population that tuberculosis is curable. All health providers should act as destigmatizers. Elimination of poverty and better health education can help eliminate tuberculosis.

069

# **THE BIOLOGICAL PROPERTIES OF INFLUENZA A VIRUS STRAINS ISOLATED IN THE ARAL SEA REGION DURING 2014-2015 EPIDEMIC SEASON**

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## **BACKGROUND-AIM**

During the 2015-2016 epidemic season 293 nasopharyngeal swabs, collected in the patient care institutions of the Aral region, were examined.

## **METHODS**

The hemagglutination activity spectrum - in the RGA, the sensitivity of HA - on the difference of HA titres before and after Heating at 56 ° for 60 min. The sensitivity of viruses to serum inhibitors was detected in RTGA with native and warm (62 ° C - 30 min) blood serum of chicken, guinea pig, rabbit. The sensitivity of viruses to different concentrations of antiviral drugs was assessed by the level of suppression of reproduction of 100 EID50 virus in chick embryos. The dose of the drug suppressing the virus titer in the RGA twice as compared to the control (without the drug) was considered inhibitory concentration (IC50).

## **RESULTS**

Virological testing of the samples enabled to isolate eight agents, which were identified with HAI, NAI and RT-PCR assays as influenza A/H1N1 viruses. Infectious activity of viruses was of 2.33-8.77 lgEID50/0.2ml. The studies on thermostability HA showed that the strains were thermostable. All isolates were inhibitor-resistant in relation to the native serums. It was found that by the hemagglutinating activity spectrum with erythrocytes of various animal species and humans, the Kazakhstan isolates actively agglutinated all kinds of red blood cells. It was established that the isolates exhibited susceptibility to Remantadine and Tamiflu, because the antiviral drugs at doses of 6.25-25 mcg/ml completely inhibited the virus reproduction in chicken embryos.

## **CONCLUSIONS**

Thereby, the strains of influenza A virus isolated during the 2014-2015 epidemic season in the Aral Sea region, according to the biological properties within the A/H1N1 subtype generally represent homogeneous group.

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**FACTORS AFFECTING COMPLIANCE WITH TUBERCULOSIS TREATMENT IN PAKISTANI PEOPLE**A. Athar.<sup>1</sup><sup>1</sup>Fatima Jinnah Medical University/Sir Ganga Ram Hospital Lahore**BACKGROUND-AIM**

Background: Tuberculosis is one of the major causes of disability and death worldwide. More than 95% of the deaths due to tuberculosis occur in low and middle income countries. Treatment defaulting is one of the major causes of failure of tuberculosis control programs in Pakistan.

Objectives: To evaluate association between various factors affecting compliance with tuberculosis treatment. Identification of factors can help improve compliance with tuberculosis treatment.

**METHODS**

Method: A case control study was carried out to gain better understanding of impact of poverty, social stigmatization, and deficient health education, poor patient knowledge about the disease, pregnancy and treatment cost. 110 people with treatment failure, enrolled as cases were interviewed. Equal number of cured people enrolled as controls were interviewed. Cases and controls were enrolled from services hospital Lahore, Gulab devi hospital Lahore and Jinnah hospital Lahore.

**RESULTS**

Results: Analysis revealed that majority of people with treatment failure were poor, they feared social stigmatization and they had deficient health education. Most of the female patients did not adhere to the treatment due to pregnancy.

**CONCLUSIONS**

Conclusion: The findings of this study reveal the urgent need for health education to convince general population that tuberculosis is curable. All health providers should act as destigmatizers. Elimination of poverty and better health education can help eliminate tuberculosis.

071

**ROTAVIRUS INFECTION IN ELDERLY. A 7-YEAR STUDY PRIOR TO THE INTRODUCTION OF ROTAVIRUS VACCINATION IN CHILDREN IN GOTHENBURG**T. Andreasson<sup>2</sup>, L. Gustavsson<sup>2</sup>, M. Andersson<sup>1</sup>, J. Westin<sup>2</sup>, M. Lindh<sup>1</sup>, L. Andersson<sup>2</sup><sup>1</sup>Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg<sup>2</sup>Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg**BACKGROUND-AIM**

Gastroenteritis caused by rotavirus has been recognized as a leading cause of diarrhea-associated morbidity and mortality in children worldwide. However, rotavirus infections in adults have not been studied to the same extent.

Our aim was to describe clinical and epidemiological characteristics in elderly hospitalized patients with rotavirus.

**METHODS**

We included all patients 60 years of age and older, hospitalized at the Sahlgrenska University Hospital in Gothenburg from September 2009 through May 2016, with a fecal swab sample positive by PCR for rotavirus. Medical records were reviewed retrospectively, and data on all-cause mortality rate, community vs. hospital onset of symptoms, ward type, date of sampling and co-morbidities was registered. Genotyping was performed by an in-house multiple PCR.

**RESULTS**

159 patients with positive rotavirus PCR were included. Hospital onset of symptoms (n=35, 22%) was less frequent than community onset (n=124, 78%). Ninety-nine (62%) of the patients with positive PCR were sampled between March through May. In the years 2013 and 2014, G2P4 was more frequent than other genotypes and found in 77% (50/65) of samples. In the other years, genotype distribution was more varied. Four patients (2,5%) died within 30 days from positive rotavirus PCR. Three of these patients were admitted to the same clinic within one month and tested positive for the same genotype of rotavirus (G2P4). All three had hospital onset of symptoms. Five additional patients at the same clinic, four with hospital onset of symptoms, were also positive for rotavirus G2P4 during the same time period. We believe these patients represent an unrecognized nosocomial outbreak with a case fatality rate of 3/8 (38%).

**CONCLUSIONS**

In the seven years preceding general rotavirus vaccination in children in Gothenburg, rotavirus infection was a notable cause of gastroenteritis-associated morbidity and hospitalization in elderly. G2P4 was the most common genotype observed. Genotyping revealed a probable nosocomial outbreak with high 30-day mortality rate among the affected patients.

072

### EVALUATION OF RHINOVIRUS RNA KINETICS IN THE GASTROINTESTINAL TRACT IN PEDIATRIC PATIENTS WITH RESPIRATORY SYNDROME

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#### BACKGROUND-AIM

The presence of Rhinovirus (HRV) RNA in stool samples highlighted recently, suggests a role of HRV as a pathogen in the gastrointestinal tract. From February to May 2014, a prospective study was conducted to verify the kinetics of HRV in the respiratory and gastrointestinal tract.

#### METHODS

Nasal swab (TNAS) and stool samples of 176 immunocompetent pediatric patients hospitalized with respiratory symptoms were analyzed with a quantitative HRV real-time RT-PCR. In the patients with TNAS positive for HRV at the first screening, TNAS and stool were collected every three days until negativization. VP2-VP4 genes were sequenced for subtyping HRV. In parallel, stool samples of 69 patients hospitalized for gastrointestinal syndrome only were tested for HRV.

#### RESULTS

HRV-RNA was detected in TNAS and stool samples of 19/176 (10.8%) patients. Two or more sequential paired samples were collected in 9/19 patients. Analysis of infection kinetics obtained comparing RNA viral load in the two biological samples showed a comparable trend. HRV-RNA in both TNAS and stool samples was detected simultaneously in 8/9 (8.8%) patients. Of 14/19 patients, HRV sequencing showed the presence of 12 HRV-A, 1 HRV-B and 1 HRV-C. The same viral strain was observed in the respiratory and gastrointestinal tract in 13/14 patients. Finally, in 69 stool samples collected from patients with gastrointestinal syndrome, HRV was negative.

#### CONCLUSIONS

In this study HRV was detected in 10.8% of both TNAS and feces in patients with a respiratory disease, while in patients with gastrointestinal symptoms, HRV RNA was never detected in stool samples. The comparable trend of HRV-RNA kinetics of the paired TNAS and stool samples demonstrated that the virus found in the gastrointestinal tract might be "non-replicating". Furthermore, HRV persistence in gastrointestinal tract following respiratory infection was never found. In 93% of the cases with sequentially TNAS-stool sample pairs, HRV strain was identical. In conclusion, it could be hypothesized that HRV detection in stools might be correlated to the passage through the gastrointestinal tract.

073

### DEVELOPMENT OF A LYOPHILISED MULTIPLEX PCR RUN CONTROL REAGENT FOR THE CLINICAL DIAGNOSES OF GASTROINTESTINAL INFECTION

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<sup>1</sup>NIBSC

#### BACKGROUND-AIM

Gastrointestinal infection is a major cause of disease worldwide. In the UK, one in five people are affected each year (1). Accurate and efficient identification of enteric pathogens is important for gastroenteritis diagnosis and treatment. Clinical diagnostic laboratories routinely use nucleic acid amplification tests (NAT)-based assays for gastrointestinal diagnosis and quality control of such assays is imperative to ensure that the reported results are indeed quality and accurate. External "in run" quality control reagents enable laboratories to monitor the quality of NAT performances. The control reagent is tested (extracted and amplified) alongside and as a clinical sample would be. When used regularly quality controls can highlight any irregularities in testing procedures and allows comparisons of testing performances between laboratories.

The National Institute of Biological Standards and Control (NIBSC) produce a range of CE-marked control reagents for NAT. Currently, these are mainly frozen liquid, single-target, whole virus preparations which include the enteric viruses Norovirus GI and Norovirus GII only. As clinical diagnostic laboratories and commercial assays are more commonly testing for multiple pathogens in a clinical sample we are taking the approach to develop a multi-analyte run control. The gastrointestinal multiplex control will be lyophilised to reduce costs by facilitating shipping and storage.

#### METHODS

A questionnaire was sent to clinical diagnostic laboratories to determine which enteric pathogens should be present in a control material. Due to the complexity of the control and the different type of pathogens in the product, freeze drying conditions and formulation were optimised.

#### RESULTS

A mixture of 19 enteric pathogens (6 virus, 3 parasite and 10 bacteria) were chosen based on the questionnaire. Optimisation of the freeze drying formulation required the cryoprotectant mannitol, in addition to the lyoprotectant trehalose, for the stability of a number of the viruses.

#### CONCLUSIONS

The availability of a gastrointestinal multiplex control for NAT will allow clinical diagnostic laboratories to effectively monitor testing performances and improve standardisation of assays in the clinical diagnostic field.

074

# **ROTAVIRUS NSP4 TRIGGERS DRP1-DEPENDENT DISRUPTION OF MITOCHONDRIAL NETWORK WHICH INITIATES INTRINSIC APOPTOTIC CASCADE**

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## **BACKGROUND-AIM**

Mitochondria are highly dynamic organelles which constantly undergo fission and fusion either to induce or inhibit apoptosis. Modulation of mitochondrial dynamics by viruses is a known phenomenon but underlying mechanism remaining to be explored. In this study mitochondrial dynamics during Rotavirus (RV) infection, was analysed.

## **METHODS**

Status of mitochondrial network dynamics during RV (SA11, Wa, OSU, A5-13) infection was checked by immunofluorescence taking COX-IV as a mitochondrial marker. Level of Drp1 and Ser 616 Drp1 in whole cell lysates and mitochondrial fractions were assessed by western blotting.

## **RESULTS**

During RV infection distinct pattern of mitochondrial fragmentation was observed in vitro. Rotaviral enterotoxin NSP4 has been shown to translocate to mitochondria and causes mitochondrial membrane depolarization, thus involvement of RV NSP4 in mitochondrial network disruption was investigated. Result revealed punctate and dispersed mitochondrial morphology in RV-NSP4 overexpressing cells. RV-NSP4 was found to induce ser-616 phosphorylation and subsequent translocation of Drp1 to mitochondria. NSP4 mediated disruption of mitochondrial network was independent of NSP4 mediated modulation of intracellular calcium ion concentration. NSP4 was found to interact with and maintain the levels of Drp1 in mitochondria during RV infection even in absence of its known adaptors Mff and Fis1. RV NSP4 induce intrinsic apoptotic pathway during late hours of RV infection via cytochrome c release from mitochondria into cytosol. Inhibition of mitochondrial fission using a specific inhibitor of Drp1 (Mdivi-1) resulted in inhibition of RV induced apoptosis as assessed by reduced cytochrome C release into cytosol and significantly low level of caspase 3 and caspase 9 cleavage in RV-SA11 infected cells treated with Mdivi-1. Mean  $\pm$  standard error (S.E.) of at least three independent biological replicates ( $n \geq 3$ ) was considered for analysis. In all tests,  $P \leq 0.05$  was considered statistically significant.

## **CONCLUSIONS**

Overall the current study focuses on RV-NSP4 mediated induction of apoptosis via Drp1 dependent mitochondrial fragmentation.

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# **ASSOCIATION OF ROTAVIRUS GENOTYPES AND HISTO-BLOOD GROUP ANTIGENS AMONG SPANISH CHILDREN**

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## **BACKGROUND-AIM**

Rotaviruses recognize histo-blood group antigens (HBGAs) as receptors or ligands for attachment to intestinal cells. Expression of the HBGA (A, B, H and Lewis antigens) in saliva and on the intestinal mucosa is driven by the FUT2 and FUT3 genes. Our aim was to evaluate the association between rotavirus genotypes isolated from children in the area of Valencia (Spain) and the HBGA (ABO blood group, H and Lewis antigens) of the patients.

## **METHODS**

A total of 133 rotavirus-infected children were recruited for this study from 2013 to 2015. Rotavirus antigens were previously detected in stool samples by immunochromatography or by EIA. Rotavirus G and P genotypes were characterized by multiplex RT-PCR following procedures standardized by the European EuroRotaNet network. Saliva samples were used to screen Lewis antigens (Lea and Leb) by ELISA and to genotype FUT2 alleles (secretor status) and ABO blood group by PCR-RFLP with the extracted host DNAs.

## **RESULTS**

Among the 133 rotavirus isolates, 132 were genotype P[8] (99.2%) and only 1 was genotype P[4] (0.7%). The detected G genotypes were G1, G2, G4, G9 and G12. Rotavirus G9P[8] was predominant (49.6%), followed by G1P[8] (20.3%) and G12P[8] (14.2%). Among the patients, the most frequently observed blood group antigens were A (45.5%) and O (39.2%). G1P[8] and G12P[8] strains mainly infected Lea-b+ individuals, whereas G9P[8] infected equally Lea-b+ and Lea+b+ patients. There were only found 2 (1.5%) nonsecretors (FUT2-) infected with G1P[8] and G1+G9P[8] strains. G9P[8] genotype mainly infected heterozygous secretors, but other genotypes equally infected heterozygous and homozygous secretor individuals.

## **CONCLUSIONS**

Rotavirus G9P[8] genotype was the most prevalent in our geographical area during the study period, infecting mainly individuals with blood groups A and O, Lewis b (Leb) antigen-positive and secretors (FUT2+). Secretor status was found to correlate with the risk of rotavirus infection. Only 2 (1.5%) nonsecretors patients were infected with rotavirus, despite the fact that 20% of our population are nonsecretors. No significant associations between rotavirus genotypes, Lewis a (Lea) antigen and homozygous/heterozygous status of the FUT2 gene were found.

076

# MONITORING HUMAN NOROVIRUS REMOVAL DURING MUNICIPAL WASTEWATER TREATMENT IN PORTUGAL AND ITS POSSIBLE IMPLICATION IN OUTBREAK PREVENTION

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## BACKGROUND-AIM

Human noroviruses (NoV) are a leading cause of waterborne illnesses. NoV infection results in high levels of virus excretion through faeces, during long periods, and consequent introduction into wastewater treatment systems. Municipal wastewater reuse has gathered special interest due to water scarcity, however, the discharge of inadequately treated sewage effluents may represent a threat to public health. Therefore, treated effluents should be monitored in order to assess the effectiveness of treatment processes in removing human viruses, such as NoV, and help prevent outbreaks through environmental exposure.

## METHODS

Fifteen Portuguese wastewater treatment plants (WWTPs), serving 26,3% of the national population, were selected for the present study. Influent (WWI) and respective effluent (WWE) samples, were collected from each WWTP, in two different seasons. After virus concentration, detection and quantification of NoV (genogroups I and II) RNA in wastewater samples was achieved by two quantitative real-time RT-PCR protocols.

## RESULTS

NoV GI RNA was detected in 10 (33%) of influent samples, with a median concentration  $2,35 \times 10^4$  GC/L. Viral genome remained detectable in 5 (50%) of the respective treated effluent (median concentration  $1,91 \times 10^4$  GC/L). NoV GII RNA was detected in 27 (90%) of the tested influent samples (median concentration  $7,99 \times 10^5$  GC/L), and remained detectable after treatment in 19 (70%) of it (median concentration in the remaining 19 WWE samples  $1,89 \times 10^5$  GC/L).

## CONCLUSIONS

The present study describes the first assessment of NoV in Portuguese wastewater systems. NoV genome was detected in 93% of the evaluated WWTP, revealing its widespread in sewage from different regions of Portugal. Moreover, the majority of studied WWTP were not able to completely remove the virus, which, by this manner will end up incorporating treated sewage and be distributed to the surrounding environment, where it may be account for source of human infection. Therefore, special attention should be done to used treatment processes, as well as to monitoring treated effluents, in order to prevent environmentally associated outbreaks.

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# ROTAVIRUS DIARRHOEA AND MOLECULAR EPIDEMIOLOGY OF ROTAVIRUSES IN SLOVENIA, 2007-2016

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## BACKGROUND-AIM

In countries with low vaccination coverage or without vaccination rotaviruses remains one of the important cause of acute diarrhoea in children 0-5 years of age. In Slovenia both rotavirus vaccines are available, but the vaccination coverage in new-borns is still low (20.7% in 2016). The aim of the work was to analyse rotavirus epidemiology and molecular characteristics after the vaccine introduction in Slovenia.

## METHODS

During the study period 2007-2016 the data on rotavirus cases was obtained from the records in the national database (National Institute of Public Health). In addition, 4222 strains from the study period were collected for genotyping of VP7 and VP4 genes according to the EuroRotaNet protocol. Maximum likelihood phylogenetic tree for the most frequent genotypes circulating during the studied years was constructed and VP7 and VP4 neutralizing epitopes of aligned amino acid sequences were analysed.

## RESULTS

According to the national database records the incidence of rotavirus diarrhoea and hospital admissions for rotaviruses were decreased for 40% throughout the seasons 2007 to 2016. The vaccination rate of new-borns was 5.5% and 10.7% in 2007 and 2008, respectively, but in the period 2009-2016, it varied between 18.4% and 26.9%. Among the most frequent genotypes G1P[8] was predominant in 2014/15 (77.9%), G2P[4] in 2015/16 (60.0%) and G4P[8] in 2009/10 (52.1%). For these genotypes, a phylogenetic clusters were observed, corresponding to the year of appearance, most evident in the VP7 phylogenetic tree of G1P[8] genotype strains. They shared 97.0-100.0% of nucleotide identity within and 92.0-97.5% between the clusters. Within the defined VP7 antigenic regions, G1 strains showed changes in 7-1a and 7-2 epitopes of VP7.

## CONCLUSIONS

Although there is a marked decrease in rotavirus disease in Slovenia, it might not be solely the effect of vaccination, as the coverage is still low. Beside the fluctuation of rotavirus genotypes, the most evident change observed during the vaccination period is the genetic shift of G1P[8] strains. Whether this was connected with the vaccine selective pressure is not clear and should be investigated in the future.

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**THE VP8\* PROTEIN FROM ROTAVIRUS P[8] GENOTYPE BINDS THE TYPE 1 PRECURSOR ANTIGEN**S. Vila-Vicent<sup>1</sup>, R. Goalbo-Rovira<sup>1</sup>, C. Santiso-Bellón<sup>1</sup>, M. Yebra<sup>2</sup>, J. Buesa<sup>1</sup>, J. Rodríguez-Díaz<sup>1</sup><sup>1</sup>Departamento de Microbiología y Ecología, Facultad de Medicina, Universidad de Valencia, Valencia, Spain<sup>2</sup>Laboratorio de Bacterias Lácticas y Probióticos, Departamento de Biotecnología de Alimentos, IATA-CSIC, Valencia, Spain**BACKGROUND-AIM**

Rotaviruses are the main cause of viral gastroenteritis in children under five years of age. Capsid protein VP4 recognizes HBGAs on the enterocytes and facilitate attachment to host cells. Trypsin cleavage of VP4 yields fragments VP8\* and VP5\*, being the VP8\* fragment the viral haemagglutinin. Human milk contains oligosaccharides (HMOs) in concentrations up to 10 to 20 g/L, comprising the third solid component of human milk. Although there are differences HMOs structures, all of them show lactose (Lac; Gal-1-4Glc) in the reducer end. That end is elongated by the addition of lacto-N-biose (LNB; Gal-1-3GlcNAc) by a 1-3 link to form type I chains and the addition of N-acetyl-lactosamine (LacNAc; Gal-1-4GlcNAc) by beta1-3/6 link to form type II chains. HMOs have similar structures to histo-blood group antigens (HBGAs). Their function remains uncertain yet.

**METHODS**

The binding ability of VP8\* recombinant rotavirus proteins to different HMOs have been analyzed. VP8\* proteins from different rotavirus genotypes were expressed in E.coli as GST-tagged proteins and purified by affinity chromatography. To analyse the binding patterns of recombinant GST::VP8\* protens to HBGAs and to some HMOs ELISA-like binding assays and surface plasmon resonance (SPR) were performed. Furthermore, binding blocking assays were carried out with soluble HMOs enzymatically produced in the laboratory including LNB and galacto-N-biose (GNB, Gal-1-3GalNAc).

**RESULTS**

VP8\* from the P[8] genotype is able to bind to the precursor disaccharide of H type 1 antigen (LNB; Gal-1-3GlcNAc). We demonstrate a new link between lacto-N-biose (LNB) and the VP8\*-P[8] genotype. This linkage is similar to the previously reported binding of the mature H type 1 antigen to VP8\* but with lower affinity as calculated by SPR. Soluble enzymatically produced LNB and GNB were able to block the binding of VP8\* both to the complete H1 antigen and to its precursor showing the potential of these HMOs as antiadhesins. Addition of LNB and GNB to milk formula could provide a new strategy for rotavirus diarrhoea treatment.

**CONCLUSIONS**

HMOs acts as an antiadhesine to pathogenic agents, mimicking host cell receptors. Therefore they may act as antiadhesins and prebiotics with healthy effects to babies.

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**MOLECULAR CHARACTERIZATION OF SAPOVIRUS INFECTION BETWEEN 2007 AND 2015 IN GERMANY**S. Niendorf<sup>1</sup>, S. Jacobsen<sup>1</sup>, J. Wolff<sup>1</sup>, M. Höhne<sup>1</sup>, T. Bock<sup>2</sup><sup>1</sup>Consultant Laboratory for Noroviruses, Unit of Viral Gastroenteritis and Hepatitis Pathogens and Enteroviruses, Department of Infectious Diseases, Robert Koch Institute, Berlin<sup>2</sup>Unit of Viral Gastroenteritis and Hepatitis Pathogens and Enteroviruses, Department of Infectious Diseases, Robert Koch Institute, Berlin**BACKGROUND-AIM**

Sapoviruses (SaV) belong to the family of Caliciviridae and are mainly associated with acute gastroenteritis (AGE) in children, although adults and elderly can be affected. SaVs can be divided into five genogroups (GI to GV), of these GI, GII, GIV and GV are known to infect humans, whereas GIII infect porcine species. SaV are recognized as etiologic agents for outbreaks and sporadic cases of AGE.

**METHODS**

Between January 2007 and December 2015 a total of 3502 stool samples of gastroenteritis outbreaks and sporadic cases were analysed for SaV infection. Retrospectively, these samples were analysed for SaV infection using RT-PCR techniques to determine the rate of SaV positive samples in the study population. Phylogenetic analyses were performed to characterize the diversity of circulating SaV in Germany.

**RESULTS**

Overall, 94 SaV positive samples (2.7%) from 48 outbreaks and 24 sporadic cases of AGE were detected. Males are more frequently infected compared to women 63.5% and 36.5%, respectively. SaV infections were more often detected during the winter season from November to March. Notably, between 2007 and 2009 a detection rate of SaV infection of 7.6% was identified. However, since 2009 the rate of SaV infection decreased and ranged between 0.8% (2011) and 2.7% (2015). The highest overall detection rate of SaV infections was determined in children of 5-9 years (7.9%), followed by children of 1-4 years (7.0%). An unexpected high positive rate was determined in adult patients (30-39 years) with 5.7%. The lowest detection rate was found in very young children (<1 year) and in elderly (>70 years) unlike to norovirus. 61/94 SaV positive samples could be genotyped. Genotype GI.2 (22.3% n=21) was predominant, followed by GI.1 (11.7% n=11), GII.3 (8.5% n=8), GIV (8.5% n=8), GII.1 (6.4% n=6) GI.6 (3.2% n=3), GV.1 (2.1% n=2), GI.3 (1.1% n=1) and GII.2 (1.1% n=1).

**CONCLUSIONS**

Our analyses have shown that SaV is a causative agent for gastroenteritis outbreaks in children and younger adults and the circulating SaV in Germany exhibit a high genomic diversity and winter seasonality.

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# A SINGLE-CENTER CLINICAL EVALUATION OF THE ARIES® NOROVIRUS ASSAY, A SAMPLE TO ANSWER, REAL-TIME PCR ASSAY FOR THE DETECTION OF NOROVIRUS IN SYMPTOMATIC SUBJECTS

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## BACKGROUND-AIM

Norovirus infection is the most common cause of gastroenteritis outbreaks worldwide. Most noroviruses that infect humans belong to genogroups GI and GII. Norovirus infections are highly contagious and often occur in closed and crowded environments such as hospitals, nursing homes or cruise ships. Accurate diagnosis of norovirus infection is important for outbreak management and epidemiological purposes. The Luminex® ARIES® Norovirus Assay is a qualitative real-time PCR assay for identification and differentiation of norovirus GI and GII in raw, unpreserved stools. The assay is used with ARIES® Systems, multiplex test systems capable of automated nucleic acid extraction and purification from a clinical sample, real-time PCR detection and data analysis in less than two hours. In this study, we assessed the clinical performance of the ARIES® Norovirus Assay in retrospectively collected, de-identified, remnant stool specimens from patients with acute gastroenteritis.

## METHODS

The performance of the ARIES® Norovirus Assay was evaluated on 200 norovirus positive and negative stool specimens collected from six sites in the US and Europe. All specimens were shipped to a single site (Luminex Madison) and tested by both the ARIES® Norovirus Assay and the reference method (Cepheid Xpert® Norovirus Assay) in a blinded fashion. Discordant results were further evaluated by bidirectional sequencing.

## RESULTS

The ARIES® Norovirus Assay Positive Percent Agreements for norovirus GI and GII were 93.3% (14/15; 95% confidence interval [CI], 70.2%-98.8%) and 98.8% (84/85; 95% CI, 93.6%-99.8%), respectively. Negative Percent Agreements of the ARIES® Norovirus Assay for norovirus GI and GII were 99.5% (184/185; 95% CI, 97.0%-99.9%) and 99.1% (114/115; 95% CI, 95.2%-99.8%), respectively. Both ARIES® false negative norovirus GI and GII specimens (as compared to Cepheid) were confirmed as negative by bidirectional sequencing. The presence of norovirus GI or GII in both ARIES® false positive specimens was not confirmed by bidirectional sequencing.

## CONCLUSIONS

The ARIES® Norovirus Assay is a sensitive and specific diagnostic tool for the safe and effective detection of norovirus GI and GII in raw stool specimens from subjects with acute gastroenteritis.

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# INVESTIGATION OF THE FREQUENCY OF ROTAVIRUS IN PATIENTS WITH ACUTE DIARRHEA

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## BACKGROUND-AIM

In this study, we aimed to investigate the prevalence of rotavirus in patients with acute diarrhea of outpatient or inpatient monitoring who admitted to Ege University Medical Faculty Hospital.

## METHODS

Between September 2016 and June 2017, stool specimens were collected from 316 (261 pediatric and 55 adult) patients with acute diarrhea. The age range of patients is between one month to 66 years (median: 6 year, mean: 11.7). Stool specimens were obtained from 108 (34.2%) outpatients and 208 (65.8%) inpatient. Stool specimens of patients with gastroenteritis were tested by a real time PCR BD MAX™ Enteric Viral Panel (BD Diagnostics, Baltimore, MD, USA) which is a diagnostic test for the qualitative detection of Norovirus (genotype I and II) and Rotavirus species in stool specimens.

## RESULTS

Rotavirus was found positive in a total of 126 (39.9%) patients with gastroenteritis, 106 of them (84.1%) were pediatric and 20 (15.9%) were adult, 46 (36.5%) were outpatients, and 80 (63.5%) were inpatients. The rate of positivity for the rotavirus in pediatric and adult groups was 40.6% (106/261), 36.4% (20/55), respectively. The age range of pediatric patients is between one month to 18 years (median: 4 years). The age range of adult patients is between 20 to 66 years (median: 34 years). The positivity rates of 0 to 5 year-old patients were 42.1%, 51.2%, 25.0%, 53.8%, 37.5%, 29.4%, respectively. Rotavirus infections were found positive 60% in January, 70.0% in February, 75.0% in March, and average 23.6 in other months.

## CONCLUSIONS

In studies conducted previously in Turkey, the rate of rotavirus positivity is found 20-70% in cases of acute diarrhea, especially first five years. In this study, rotavirus were identified approximately 40% of patients with gastroenteritis. Rotavirus was detected most frequently in 0-1 (46.6%) age group. Rotavirus infections were detected from more inpatients than outpatients. When the monthly distribution of rotavirus positivity is evaluated, it is seen that the highest rate was in March. The reason why for the highest rate of rotavirus positivity in March could be explained by the fact that March 2017 is much colder than the seasonal norms.

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### INVESTIGATION OF ENTEROVIRUSES FROM CEREBROSPINAL FLUID SAMPLES IN ASEPTIC MENINGITIS PATIENTS

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#### BACKGROUND-AIM

Enteroviruses are the most frequent viral agent of aseptic meningitis. It is difficult to diagnose enteroviral infections of the central nervous system by conventional methods and mortality is high. Although enterovirus which is transmitted with fecal-oral and respiratory has not an effective antiviral treatment and there is no vaccine against the majority of serotypes. Real-time polymerase chain reaction (RT-PCR) test can be used to detect viruses more precisely, quickly and easily than viral cell cultures. In this study, it was aimed to retrospectively evaluate the results of enterovirus (EV) RT-PCR test in cerebrospinal fluid (CSF) specimens of patients with pre-diagnosed aseptic meningitis.

#### METHODS

EV RNA was investigated in 620 CSF samples of 572 patients from emergency room, clinics and outpatient clinics at Central Laboratory of Akdeniz University Hospital, between January 2014 to January 2017. Nucleic acid extraction was performed using the EZ1 Virus Mini Kit v2.0 (Qiagen, Germany), followed by Argene Enterovirus R-gene (Biomerieux, France) and Progenie RealCycler EVPA (Progenie, Spain) kits were used for RT-PCR assay.

#### RESULTS

In this study 423 (73.95%) of the patients were male, 149 were female (26.05%) and the mean age was 18.8 ( $\pm$  19.2). EV RNA was detected in the CSF sample of 15 (2.62%) patients (9 female, 6 male). The mean age of the patients who were positive was 12 ( $\pm$  11.1) and 13 were children age group. Three positive samples were identified in winter, two in spring, four in summer and six in autumn. Although there was an increase in the demand for test according to years, there was no increase in the rate of detection of positivity. EV infections which were affecting the central nervous system were more frequent in the pediatric age group. Patients who were found to have positivity have mostly applied in the summer and autumn.

#### CONCLUSIONS

EV should be considered as the causative agent in patients who have viral central nervous system infection is suspected with nonspecific clinical findings. It is possible to detect EV early and early in CSF specimens with molecular methods.

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### INVESTIGATION OF ENTEROVIRUSES, HERPESVIRUSES (TYPES 1,2,6,7), FLAVIVIRUSES AND PHLEBOVIRUSES BY POLYMERASE CHAIN REACTION IN PATIENTS WITH ATYPICAL MENINGITIS/ENCEPHALITIS

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#### BACKGROUND-AIM

During routine diagnostic services, viral agents causing atypical meningitis/encephalitis mostly remain obscure. The aim of this study was to investigate the impact of herpesviruses (types 1,2,6,7), enteroviruses, flaviviruses and phleboviruses in patients with atypical meningitis/encephalitis.

#### METHODS

Paediatric cases (age:0-18) that presented with neurological complaints at two hospitals in Ankara with the etiologic agent undetermined via routine diagnostic assays were evaluated. One hundred cerebrospinal fluid (CSF) specimens were collected and processed via. QIAamp® Viral RNA Mini Kit (Qiagen,Germany) and RevertAid First Strand cDNA Synthesis Kit (ThermoFisher SCIENTIFIC, USA). A commercial real time PCR assay (LightCycler® HSV1/2 Qual Kit, Roche, Germany) was employed for Herpes Simplex virus (HSV) type 1/2 detection. Enteroviruses, HHV6, HHV7, flaviviruses and phleboviruses were investigated by in house PCR methods.

#### RESULTS

The patients comprised 61 male and 39 females. A total of 6 specimens (6%) were positive for viral agents. Four (4%) of the positive samples were HHV6 and 2 (2%) of them were HSV1. The other agents (HSV2, HHV7, EV, flaviviruses, phleboviruses) tested were negative. Two of HHV6 DNA positive patients were belongs to 1-6 months old age group, 1 of was 13-36 months old and the other was 4-6 years old. Sixty three percent (63/100) of the patients had meningitis prediagnosis, 28% of them had viral encephalitis prediagnosis and the rest 9% had some prediagnosis such as meningoencephalitis, shunt infection, varicella infection, acute flaccid paralysis. HHV6 was detected in 3 patients with meningitis, in 1 patient with viral encephalitis and HSV1 was detected in 2 patients with viral encephalitis.

#### CONCLUSIONS

Many viral agents can cause central nervous system (CNS) infections and detectable number of cases is low. Although HSV1 is the most common agent in viral encephalitis cases, HHV6 is a rare agent. Thus, these viral agents that might have a role in CNS infections, should be considered in diagnostic algorithms.

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# CAN GENETIC DIFFERENCES BETWEEN EV-A71 GENOTYPE C2 AND C4 STRAINS FROM PATIENTS WITH CNS AFFECTION VS. PATIENTS WITH Milder DISEASE EXPLAIN THE SEVERITY OF THE INFECTION?

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## BACKGROUND-AIM

Enterovirus A71 (EV-A71) is a common cause of Hand-Foot- and Mouth- disease, but has also been associated with severe neurological disease and fatalities. A number of recent studies have identified genetic motifs as potential determinants/markers of neurovirulence for genotype C4. In this study, cultured viruses from patients with neurological or non-neurological EV-A71 infection were investigated for the presence of such determinants.

## METHODS

Cultured viruses from 28 patients with EV-A71 genotype C2 (n=9, 4 with CNS affection) or genotype C4 (n=19, 9 with CNS affection) infection were included. Whole genome sequencing was carried out using SMART chemistry for library preparation and run on an Illumina MiSeq. Reads were assembled using CLC Genomics Workbench, and consensus sequences imported into SSE v1.2 for alignment with reference sequences. Maximum likelihood phylogenetic analysis was carried out using MEGA6. Nucleotide and amino acid sequences were analyzed for the presence of motifs associated with virulence.

## RESULTS

Near complete genomes were achieved for all samples. There was no phylogenetic clustering of neurological vs. non-neurological strains for either genotype C2 or C4. We found no association between any of the previously reported genetic determinants for neurovirulence with Danish neurological C2 or C4 strains as compared to non-neurological strains. Two of the Danish neurological C2 strains shared two amino acid substitutions, one in the 5' UTR and one in 3Dpol. One published amino acid substitution in 2C was detected in one genotype C4 neurological strain, and one nucleotide substitution in the 5' UTR was detected in 3 neurological and 2 non-neurological C4 strains. There were no common nucleotide or amino acid substitutions that distinguished any of the Danish neurological C4 strains from non-neurological C4 strains.

## CONCLUSIONS

In this study, we were not able to identify any of the previously reported motifs associated with neurovirulence in Danish EV-A71 strains. We also did not identify any additional motifs as potential markers for neurovirulence. Our study therefore suggests that severity of EV-A71 disease is determined by host-specific factors rather than virus-associated characteristics.

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# OUTBREAK OF ENTEROVIRUS D68 DIAGNOSED IN TRONDHEIM, NORWAY, AUGUST 2016 TO JANUARY 2017

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## BACKGROUND-AIM

EV-D68 was first described in 1962 and has been associated with a variety of clinical manifestations, especially in children. In 2014 EV-D68 emerged worldwide and in some cases caused severe respiratory as well as neurological symptoms, including acute flaccid paralysis (AFP). In August 2016 there was an outbreak of respiratory infections caused by EV-68 in our part of Norway, with a peak during September. No EV-D68 was diagnosed after January 2017.

## METHODS

Detection of EV-D68 was performed by a specific real-time PCR. During the period August 2016 to January 2017, 1525 specimens were tested, and in addition, EV-D68 was cultured in Vero cells and sequenced from respiratory samples of a nine years old girl who developed AFP.

## RESULTS

EV-D68 RNA was detected in 165 respiratory specimens from 160 patients. 22 CSF specimens tested negative, including the patient with AFP. 44 other specimens, mainly stool samples, were also negative. All patients had respiratory symptoms, and three of them were admitted to intensive care units. The patient with AFP developed asymmetric motoric paresis involving legs and arms and she needed ventilation for several days before she gradually recovered. MRI showed increased signal on T2 in central gray matter from C3 to conus compatible with myelitis, but there was no detectable sensory loss.

## CONCLUSIONS

During the period from August 2016 to January 2017, there was an extensive outbreak of EV-D68 infections in Norway. One of 160 patients developed serious AFP in addition to severe respiratory symptoms, virus could not be detected in CSF or fecal specimens in any patient. This is in accordance with other observations seen in patients with AFP caused by EV-D68.

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# A CASE OF ACUTE FLACCID MYELITIS ASSOCIATED TO ENTEROVIRUS D68 IN AN ADULT WITH IATROGENIC IMMUNOSUPPRESSION IN ITALY

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## BACKGROUND-AIM

A rare Enterovirus serotype D68 (EV-D68) has been recently upsurged as an emerging pathogen; it typically causes mild respiratory illness, but occasionally may progress to more severe clinical syndromes.

In 2014, a large EV-D68 respiratory outbreak occurred in North-America and Europe and an apparent increased of incidence of Acute Flaccid Myelitis (AFM) has been reported, although viral genome was detected exclusively in respiratory specimens.

We report a fatal case of EV-D68 infection in an adult woman, hematopoietic stem cells transplant recipient, who developed AFM.

## METHODS

In October 2016 an adult woman with a clinical history of follicular Non-Hodgkin B-Lymphoma (B-NHL) was admitted to the Hematology Emergency Unit of Policlinico Umberto I, Rome (Italy), presenting sudden acute weakness of arm, neck and head.

EV was detected in Cerebrospinal Fluid (CSF) by FilmArray ME and confirmed by RT-qPCR in CSF, oro-pharyngeal swab and stool.

Screening by RT-qPCR of CSF for other known neurotropic viruses, such as Flaviviruses, CMV, EBV, HSV1 and 2, VZV, yielded negative results

The EVs genus/species identification was determined by analyzing the partial-length of the 5'UTR and VP1 sequences with the web-based open-access Enterovirus Genotyping Tool Version 0.1

Phylogenetic analysis was performed using the ML method, HKY+G1 Model. Phylogenetic analysis of VP1 was carried out in a context of 918 EV-D68 worldwide sequences.

## RESULTS

The present findings support the role of EV-D68 in the etiology of AFM, adding to the scarce number of cases where the presence of the virus genome was evidenced in CSF and not only in respiratory specimens. In addition, to our knowledge, this is the first evidence of an EV-D68-associated with AFM in an adult in Italy.

The phylogenetic analysis shows that the virus clustered with the recently described subclade B3, which includes sequences involved in the 2016 Netherlands outbreak, and is closed to subclade B1, mainly formed by EV-D68 strains detected in patients who developed AFM during North-American outbreak in 2014.

## CONCLUSIONS

In conclusion, immunocompromised adults, like healthy young children, may be at risk of EV-D68-related neurological complications and highlights the importance of including EV-D68 in the diagnostic strategy for immunocompromised patients

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# MOLECULAR EPIDEMIOLOGY OF NON-POLIO ENTEROVIRUS IN THE REGION OF LAZIO FROM 2012 TO TODAY

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## BACKGROUND-AIM

Enteroviruses (EVs) are the most common worldwide circulating viruses which can cause a broad spectrum of clinical illness and the most frequent etiological agent of aseptic meningitis in children. In the majority of cases the infection only manifests flu-like symptoms- if any- and is self-limiting, thus leading to an underestimation of the infection in the population. The genus includes more than 100 different serotype and recently some of them, as EV-71 and EV-D68, are emerging like viruses that can lead to a flaccid paralysis comparable to poliomyelitis (defined AFP/AFM).

In this study, we investigated the molecular epidemiology of EVs in Lazio from 2012 to today, using a molecular method able to genotype the EVs.

## METHODS

The study identified 60 patients with EV infections since 2012 to today. Enteroviral-RNA was detected in cerebrospinal fluids (CSF), respiratory specimens and stools by commercial One-Step qRT-PCR method. The EVs typing was determined by analyzing the partial-length of the 5'UTR and VP1 sequences with the web-based open-access Enterovirus Genotyping Tool (V 0.1). The sequences were aligned by the Clustal algorithm and phylogenetically analysed using the MEGA 7 software package.

## RESULTS

From January 2012 to January 2017, the Laboratory of Virology INMI have been analyzed for the diagnosis of EV infection 1213 patients. The results show that 5% of the patients result positive, mostly of them are male (58,3 %) and belong to the age group of 25-49 years although the largest % of positive samples is related to patients younger than 14 years old (19.3%).

Most (83%) of molecularly characterized EVs belongs to species EV B (5 serotype of echovirus and 3 of Cox), 14,9% EV A (Cox A16 and A6) and 2,1% EV D (D68); we have found no EV C.

The phylogenetic analysis reveals 3 small outbreaks (E11 and E30 in 2013; E9 in 2016) and the co-circulation of different serotype in Lazio that could be attributed to a genetic drift of EVs endemic strains leading to a microepidemic circulation pattern of strains, as highlighted for E6.

## CONCLUSIONS

In conclusion, our genotyping approach of EVs strains from clinical specimens, in addition to phylogenesis proves a reliable method to rapidly identify the emergence of new EV variants and epidemic strains.

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### ENTEROVIRUS D68 DETECTION IN RESPIRATORY SAMPLES FROM THE 2016 OUTBREAK AND A PEDIATRIC MENINGO-ENCEPHALITIS COHORT

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#### BACKGROUND-AIM

Recent enterovirus (EV) D68 outbreaks have been associated with neurological symptoms in healthy children. These EV-D68 associated central nervous system (CNS) symptoms include acute flaccid paralysis/myelitis (AFP/M) and encephalitis or meningitis. In patients with a positive respiratory sample for EV-D68, AFP/M incidence rates of 0.7-4.1 % have been reported. EV-D68 is mainly detected in respiratory samples, and as most laboratories do not routinely screen for EVs in respiratory specimens, EV-D68 can easily be missed.

#### METHODS

In order to test whether our routine diagnostics would miss cases of EV-D68, we evaluated the diagnostic testing in respiratory samples. Our routine diagnostics on all respiratory samples is a multiplex RT-PCR including rhinovirus (RV) and EV. EV-positive samples are genotyped, and an EV-D68 specific PCR is performed in the event of EV and RV positivity. In this study, with a specific EV-D68 PCR, we evaluated respiratory samples from pediatric and adult patients (n=153) of whom at least one respiratory sample was available in the 2016 EV-D68 outbreak period (21 June to 21 September 2016). To evaluate whether EV-D68 had been missed in neurological cases, we subsequently screened respiratory samples (n=68) from a pediatric meningo-encephalitis (ME) cohort (the PACEM study, METC 2014\_290) by a specific EV-D68 PCR.

#### RESULTS

In the 153 respiratory samples that were available during the 2016 outbreak we detected one patient with a positive EV-D68 respiratory sample, in addition to six EV-D68 positive patients detected with routine diagnostics. This case was previously tested positive for RV (but not EV), boca- and parainfluenza type 3 virus in our routine diagnostics. In the 2016 outbreak and pediatric ME cohort no EV-D68 associated neurological cases were identified.

#### CONCLUSIONS

With our routine diagnostic techniques we have the technical possibilities to detect EV-D68, especially when the specific EV-D68 PCR is used in case of a positive RV sample. In the 2016 outbreak and ME cohort no EV-D68 CNS cases were detected. This could be explained by the low incidence of CNS EV-D68 cases. Another explanation might be a low awareness among clinicians to test for EV-D68 as a cause for AFP/M in respiratory samples, and this may result in an underreported incidence of EV-D68 associated AFP/M cases.

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### MOLECULAR EPIDEMIOLOGY OF ENTEROVIRUSES CSF INFECTIONS IN ISRAEL, 2013-2017

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#### BACKGROUND-AIM

Enteroviruses (EVs) are single stranded RNA virus, which belongs to the Picornaviridae family. There are more than 100 non-poliovirus enterovirus distinct genotypes, known to cause human infections, of which meningitis is one of the most severe clinical presentation. Available robust molecular real time PCR (q-RT-PCR) diagnostic assays allowed for the detection of EVs genome in patient samples. However, sequencing EVs VP1 capsid region is crucial for determining EVs genotypes. In this study, we report our algorithm for detecting and typing EVs from patient samples and how it allowed to identify three different EVs genotype specific outbreaks between the years 2013 until May 2017.

#### METHODS

Patient CSF samples (N = 4,209) collected between January 2013 and May 2017 were tested by laboratory developed and validated q-RT-PCR assay for EV 5'UTR and MS2 bacteriophage was used as an internal control for the assay performance. EV positive CSF patient samples (10%) were subjected for VP1 sequencing using the CDC protocols.

#### RESULTS

Of the 4,209 patient CSF samples, 548 (13%) were positive for EV. The EV percent positivity varied between 8.3% in 2013 and 27% in 2017 (up to May). The majority of the patients infected with EV where in the age group 6-10 years of age. The monthly EV epidemiology curve showed three peaks (August 2015, March 2016 and March 2017). Typing EV revealed that throughout the study period, 33 different EVs genotypes caused the patients symptoms. However, what was important was the detection of Echovirus 5 and Echovirus 9 outbreak in August 2015, Echovirus 30 outbreak in March 2016 and Echovirus 18 outbreak in March 2017. Typing the EVs allowed the health care providers to produce a clear EV meningitis outbreak "Case Definition" as each EV outbreak had a different target age group and gender. For Echovirus 30 outbreak in 2016, infant and females in age group 20 to 40 years of age were most affected groups. However, the Echovirus 18 outbreak in 2017 was mainly seen in males less than 40 years of age.

#### CONCLUSIONS

Our study is the first to report the epidemiology of EV meningitis in Israel since 2013 and its results supports the need for performing EV molecular typing in order to detect and characterize EV genotype related outbreaks.

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# INVESTIGATION OF ENTEROVIRUS RNA IN PATIENTS WITH CENTRAL NERVOUS SYSTEM INFECTIONS

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## BACKGROUND-AIM

Although enterovirus (EV) infections are frequently either asymptomatic or causing only mild febrile illness, non-polio enteroviruses (NPEV) are also the most common viral pathogens responsible for aseptic meningitis in patients of all age groups. EVs are the pathogens associated most commonly with acute meningitis worldwide and can cause sporadic cases, outbreaks, and epidemics. Molecular techniques based on the amplification by reverse transcription polymerase chain reaction (RT-PCR) of conserved parts of the EV genome are highly sensitive and currently the golden standard to detect small RNA amounts of enteroviral RNA directly from clinical samples. The purpose of our study was to investigate EV RNA via an in house assay, in patients with neurological symptoms.

## METHODS

The study was undertaken in Ankara province, Central Anatolia, Turkey. Patients, clinically diagnosed with febrile disease and/or central nervous system infections of presumed viral aetiology, were enrolled in the study with informed consent. Sera and/or cerebrospinal fluid specimens were collected from 38 adult patients, attended to Hacettepe University Hospital from June 2012 to March 2013. Clinical history and follow-up, physical examination and standard laboratory findings of the patients were recorded. Nucleic acid extraction and complementary DNA synthesis was performed via commercially available assays. EV screening was performed via a set of novel primers, designed to amplify a 193-basepair section of the 5'-untranslated region of enteroviruses. The assay was optimized using cell culture grown Cocksackie virus B6 (Schmitt isolate).

## RESULTS

In all patients, bacterial, mycobacterial and fungal cultures were negative, as well as PCR for herpes simplex virus (HSV) types 1/2. Cerebrospinal fluid specimens of 33 patients were available for EV detection. PCR results of all samples were negative for EV.

## CONCLUSIONS

Although EV is not a frequently-reported agent of central nervous system infections in Turkey, it should be included in the diagnostic workup of relevant cases.

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# ACUTE FLACCID MYELITIS (AFM) ASSOCIATED WITH ENTEROVIRUS D68 (EVD68) INFECTION, 2016

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## BACKGROUND-AIM

A 4-year-old child was referred to pediatric emergency department of the Sant'Anna Hospital (Como, Italy) because of pharyngitis with fever (38.8°C), mild headache and neck stiffness without any other meningeal sign, normal deep tendon reflexes.

## METHODS

Leucocyte count was 14x10<sup>9</sup>cells/l (68% neutrophilocytes), CRP was negative. The following morning he presented with muscle weakness, mainly proximal, in left arm. Cerebral magnetic resonance imaging (MRI) showed normal findings. Lumbar puncture revealed pleocytosis (80x10<sup>6</sup>cells/l), proteinorachia (76 mg/dl) and increased immunoglobulin G index without oligoclonal bands. In the evening, flaccid paralysis in upper extremities and absent deep tendon reflexes with normal tactile and thermic sensibility were evident. An acute myelitis in the anterior horns of the spinal cord was suspected. Spinal MRI scans confirmed edema of mainly grey matter in a longitudinal pattern consistent with a diagnosis of AFM. Methylprednisolone (30 mg/kg/die) administration was started and the child was admitted to intensive care unit (ICU).

## RESULTS

Nasal swab and nasopharyngeal aspirate specimen tested positive for EVD68 detection by real-time RT-PCR, serum and CSF were negative. The EVD68 sequence analysis revealed the presence of an EVD68 belonging to lineage B3, closely related to 2016 Dutch and Swedish EVD68-AFM cases. The child was transferred to a pediatric ICU where presented a respiratory failure and mechanical ventilation (MV) was started and then, in just over a week, tracheotomy was performed. Nutrition was assured through percutaneous endoscopic gastrostomy (PEG). After 1 month all the signs and symptoms of AFM were reduced and the results of MRI showed normal findings. Ten months later, the child feeds without PEG but unfortunately he still needs MV and has only a slight improvement in the flaccid paralysis.

## CONCLUSIONS

This case shares striking similarities with others previously described, which allowed the suspect diagnosis. The inability to detect EVD68 in CSF is not surprising since reported rates of CSF detection for known neurotropic EVs (Poliovirus, EVA71) are as low as 0-5%. This clinical report suggests including respiratory samples search for EVD68 in the panel of examinations recommended in case of AFM and paralysis.

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# **EVALUATION OF A LYOPHILISED MULTIPLEX CONTROL MATERIAL FOR DIAGNOSIS OF SYNDROMIC TESTING OF MENINGITIS BY NUCLEIC ACID TECHNOLOGY (NAT)**

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## **BACKGROUND-AIM**

Meningitis is a life threatening disease, especially in children. Clinical presentation of the infection with any aetiological agent of meningitis results in a similar symptomatology and clinical diagnostic laboratories are increasingly moving towards a 'syndromic approach' for the simultaneous testing of possible meningitis causative agents. In order to provide suitable working run reagents for syndromic testing, at the National Institute of Biological Standards and Control (NIBSC), an IVD-CE marked multiplex working run reagent for Nucleic Acid Technologies (NAT) for the diagnosis of meningitis has been produced.

## **METHODS**

A total of 10 viral and 3 bacterial aetiological agents of meningitis were selected. Whole viral and bacterial agents were lyophilised in a universal matrix of 10mM Tris-HCl (pH: 7.4) and 0.5 mM EDTA and several excipients named sorbitol, mannitol and trehalose were evaluated for their performance as an optimal lyophilisation matrix. Selection of final lyophilisation matrix was based on post-lyophilisation stability measured as inter-vial homogeneity for each target and cosmetic properties of the lyophilisation cake.

The performance of the multiplex working run reagent was evaluated in several multiplex commercial assays available for the diagnosis of meningitis including external collaborators under routine conditions for clinical diagnostic laboratories.

## **RESULTS**

A combination of 2% trehalose and 4% mannitol was selected as the optimal lyophilisation matrix. Inter-vial homogeneity indicates that the product is homogeneous and stable for a minimum of a week period.

Analysis of the performance of the multiplex reagent in commercial assays produced satisfactory results, indicating that the product is suitable to use with IVD-CE marked meningitis multiplex commercial assays.

## **CONCLUSIONS**

An IVD-CE marked lyophilised multiplex working run control for the diagnosis of meningitis has been produced. The presence of whole pathogens resembling a clinical sample permits the reagent to be employed at all stages of clinical diagnosis such extraction and amplification. The observed performance in commercial kits suggest that represents an appropriate tool for the assessment of quality in the syndromic detection of meningitis.

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# **PRODUCTION AND EVALUATION OF A LYOPHILISED CONTROL MATERIAL FOR DIAGNOSIS OF ENTEROVIRUS ED-71 AND ED-68 INFECTIONS BY NUCLEIC ACID TECHNOLOGY (NAT)**

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## **BACKGROUND-AIM**

Enteroviruses are implicated in a diverse set of clinical manifestations ranging from asymptomatic carriers to severe neurological complications. Recent attention has focused in enterovirus ED-68 and enterovirus ED-71 for being implicated in neurological symptomatology resulting in an increased interest in unravelling the epidemiology of these particular enteroviruses.

The National Institute of Biological Standards and Control (NIBSC) produces biological standards which contribute to the monitoring of quality standards in clinical diagnostic laboratories. With the increased focus in the surveillance of non-polio enterovirus neurological complications, NIBSC is currently producing two IVD-CE marked working run reagents for Nucleic Acid Technology (NAT) to aid in the molecular detection of enterovirus ED-71 and ED-68.

## **METHODS**

ED-71 and ED-68 viral isolates from clinical specimens were fully characterised by NGS and selected as a candidates for a working run reagent. Each candidate was lyophilised in a universal matrix of 10mM Tris-HCl (pH: 7.4)- 0.5 mM EDTA and 2% trehalose. Immediate and accelerated degradation studies were conducted to evaluate post-lyophilisation stability employing both, the pan-enterovirus real time qPCR and VP1 for typing purposes.

## **RESULTS**

Comparison between pre- and post-lyophilization stages showed that no target loss occurs during the lyophilisation process and post-lyophilization stability assessed as inter-vial heterogeneity indicates adequate levels of intra-batch homogeneity.

Performance evaluation was conducted in-house employing available commercial kits. Further product validation by a collaborative study to evaluate performance under routine clinical laboratory conditions is currently under preparation.

## **CONCLUSIONS**

Two lyophilised CE-marked working run control reagents for NAT technologies had been produced for aiding in the molecular diagnosis of enterovirus ED-71 and ED-68. An advantage of the lyophilisation matrix is that ensures stability of the product at ambient temperature for logistic purposes. Furthermore, run control reagents are present as whole viral particles, therefore could be employed as a double control for the extraction as well as amplification stages, mimicking clinical samples and producing a full quality control assessment.

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# **CHARACTERIZATION OF HPV16-SPECIFIC EFFECTOR AND CENTRAL MEMORY T-CELL RESPONSES IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA (OSCC) PATIENTS AND HEALTHY VOLUNTEERS**

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## **BACKGROUND-AIM**

Oropharyngeal squamous cell carcinoma (OSCC) rate is increasing over time with 70% of HPV16-related cases. HPV16-specific immune response might exert a role in cancer progression. The aim of this study was to characterize effector and central memory HPV16-specific T-cell responses in OSCC patients and healthy volunteers.

## **METHODS**

Forty patients with OSCC were evaluated; of these, 16 (40%) had biopsy-proven HPV16-related OSCC and 24 (60%) had HPV16-unrelated OSCC. Additionally, 33 healthy volunteers were analyzed; of these, 7 (21,2%) have been vaccinated for high-risk HPV types 5-10 years earlier. HPV16-specific T-cell responses were quantified by using peptide-based standard and cultured ELISPOT assays (quantifying effector memory and central memory T-cells, respectively). PBMC were stimulated with HPV16-specific L1, E6 and E7 peptide pools and IFN $\gamma$ -producing T cells were quantified after short-term stimulation and after their expansion during a 10-day culture. HPV16-specific antibodies were quantified by neutralization assay.

## **RESULTS**

No difference was found in HPV16-specific effector memory T-cell response between patients with HPV16-related OSCC and patients with HPV16-unrelated OSCC. Although a marked L1 and E6-specific central memory T-cell responses were detected in the first group of patients ( $p=0.0383$  and  $p=0.0390$ , respectively), no difference was observed in E7-specific central memory T-cell response ( $p=0.1817$ ). Both HPV16-specific effector and central memory T-cell responses were significantly higher in HPV16-seropositive volunteers compared to HPV16-seronegative volunteers. A deficient E7-specific central memory T-cell response was evidenced in HPV16-positive OSCC patients compared to HPV16-seropositive healthy volunteers (median 49 [IQR 5.5-547] and 1258 [IQR 175-2970] net spots/million PBMC, respectively;  $p=0.0393$ ), as well as L1-specific central memory T-cell response (median 1277 [IQR 158.5-3248] and 2805 [IQR 1294-5278] net spots/million PBMC, respectively;  $p=0.0466$ ).

## **CONCLUSIONS**

The role of E7-specific memory T-cell response may be crucial. Longitudinal studies of HPV16-specific T-cell responses in OSCC patients after treatment might help to identify immunological markers of better prognosis in OSCC patients with HPV-related cancer.

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### HPV16 E6 AND E7 UPREGULATE THE EPIDERMAL GROWTH FACTOR RECEPTOR AND THE HISTONE LYSINE DEMETHYLASE KDM2B THROUGH THE C-MYC/MIR-146A-5P AXYS

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#### BACKGROUND-AIM

Aim of this study was the investigation of miRNA changes induced by HPV E6 and E7 oncogenes in human keratinocytes and HPV-related cancers and their role in tumorigenesis.

#### METHODS

Microarray and qRT-PCR analysis of miRNA expression; site-directed mutagenesis of the miR-146a promoter and luciferase reporter assays; Western-blot analysis and 3'UTR reporter assay to validate miRNA targets; gene silencing by shRNA; cell proliferation, migration, and clonogenic assays; IHC, cISH and FISH.

#### RESULTS

Microarray analysis showed that E6 and E7 of high-risk HPV16, but not low-risk HPV6, altered the expression of a set of miRNAs in human foreskin keratinocytes. Specifically, E6 of HPV16 down-regulated miR-34a and miR-146a-5p, while E7 up-regulated miR-16, miR-34b, miR-34c, and miR-486-5p. These changes were confirmed in cervical carcinoma cell lines, in high-risk HPV-positive cervical specimens, penile cancers, and laryngeal squamous cell carcinomas. Among altered miRNAs, miR-146a-5p was selected for functional evaluation. Site-directed mutagenesis of a c-MYC binding site in the miR-146a promoter led to increased promoter/luciferase reporter activity in HPV16 E6/E7-positive cells, suggesting that miR-146a-5p down-regulation was mediated by the transcription repressor c-MYC. Overexpression of miR-146a-5p significantly inhibited proliferation and migration of keratinocytes and cervical cancer cells. The epidermal growth factor receptor (EGFR) was confirmed to be a target for miR-146a-5p and was found to be significantly overexpressed in high-risk HPV-positive cancers. The histone demethylase KDM2B was validated as a new direct target of miR-146a-5p and two putative binding sites for miR-146a-5p were identified in its 3'UTR sequence. KDM2B was overexpressed in HPV16 E6/E7-positive keratinocytes, in cervical cancer cell lines, and in a subset of invasive cervical carcinomas and HPV-positive laryngeal squamous cell carcinomas. In these tumors, KDM2B overexpression was associated with c-MYC copy number gain. Silencing of KDM2B inhibited proliferation of cervical cancer cells.

#### CONCLUSIONS

E6 and, less efficiently, E7 of high-risk HPV16 up-regulated EGFR and KDM2B expression in human keratinocytes through a pathway involving overexpression of c-MYC, which in turn downregulated miR-146a-5p.

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### ASSESSING THE ONCOGENICITY OF THE ASIAN PREVALENT HUMAN PAPILLOMAVIRUS TYPE 58 E7 VARIANTS

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#### BACKGROUND-AIM

Persistent infection with human papillomavirus (HPV) has been identified as the main etiological cause of cervical cancer. Interestingly, epidemiological studies have recurrently pinpointed an unexpected high prevalence of HPV-58 in East Asia. The underlying reason of this geographical/ethnic predilection in disease attribution remains unexplored.

#### METHODS

Our previous epidemiological studies identified an Asian prevalent HPV-58 E7 variant, designated as V1, to be significantly associated with higher cervical cancer risks. The current study compared the oncogenic potential of V1 and other HPV-58 E7 variants using various functional and molecular assays.

#### RESULTS

Upon HPV-58 E7 V1 overexpression, primary baby rat kidney cells showed an increased colony forming ability ( $P < 0.05$ ), implicating its higher immortalizing power. Further functional characterization revealed that HPV-58 E7 V1 conferred an analogous higher colony number in soft agar ( $P < 0.001$ ), which indicates its higher transforming potential. The oncogenic properties of HPV-58 E7 V1 was subsequently affirmed by a stronger degradation ability on exogenous pRb than the other variants ( $P < 0.001$ ), which is the major effective protein of E7 oncogenesis. Other properties of HPV-58 E7, including subcellular localization and protein stability were also characterized.

#### CONCLUSIONS

HPV-58 E7 V1 exerted higher oncogenic potential in immortalizing and transforming primary cells, possibly through exerting a strong degradation ability to pRb. Our findings not only explain, at least in part, the high prevalence of HPV-58 in Asia, but also help to devise new strategies for HPV surveillance and therapeutic management, particularly in East Asia.

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### MERKEL CELL POLYOMAVIRUS IN SECONDARY LYMPHOID TISSUES FROM ITALIAN IMMUNOCOMPETENT CHILDREN

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#### BACKGROUND-AIM

Merkel cell polyomavirus (MCPyV) is associated with Merkel cell carcinoma and chronic lymphocytic leukemia. Immunologic studies have reported MCPyV antibodies in as many as 80% of healthy blood donors and viral DNA was recovered in the buffy coats suggesting latent/persistent state in the immune competent host. Because human polyomavirus DNAs have been detected in tonsillar tissues, the high respiratory tract has been proposed as one of the entry portals for these viral agents. The aim of this study was to estimate the burden of MCPyV infection in Italian immunocompetent children (0-17 years) considering seroprevalence and viral sequences load in the secondary lymphoid organs.

#### METHODS

Seroprevalence was tested by a multiplex antibody detection system based on the fluorescent beads technology, in combination with a glutathione S-transferase (GST) capture enzyme-linked immunoassorbent assay method, in 182 immunocompetent children (0-17 years) undergoing surgery for the ablation of adenoids/tonsils.

Sequences of MCPyV Tag regions were explored in the secondary lymphoid tissues by quantitative Real Time PCR and characterized by sequencing.

#### RESULTS

MCPyV seroprevalence was of 34% in children from 0 to 3 years, 62% in children from 4 to 7 years and 41% in those from 8 to 17 years.

Although with a low viral load (<101 copy number/reaction) MCPyV was found in 6% of tonsils and in 2% of adenoids suggesting that the virus may initially hit the first respiratory tract, and then reach the latency sites. Sequencing analysis identified the European/Italian MKL-1 strain.

#### CONCLUSIONS

This study reports additional insights on the serological evidence of exposure to MCPyV during early childhood and suggests a role of the secondary lymphoid cells as a latent/persistent site, source of MCPyV horizontal infection in the human population.

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### HIGH/LOW RISK HPV GENOTYPES IN ANAL BRUSHING FROM HIV POSITIVE AND NEGATIVE MEN: PREVALENCE, PERSISTENCE AND GENETIC VARIABILITY OF L1, E5, E6, E7 AND LCR SEQUENCES

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#### BACKGROUND-AIM

Anal squamous cell carcinomas are strongly associated with high-risk (HR) HPV. Few data are available on the prevalence of anal infections with specific HPV genotypes and factors influencing anal HPV persistence and progression in HIV+ individuals. Moreover, differently from cervical cancer, the presence of low-risk (LR) HPVs has occasionally been reported in anogenital cancers. The aim of this prospective study is to provide epidemiological data on anal HPV infection, especially comparing HR/LR genotype distribution and HPV infection persistence in HIV+ and HIV- men.

#### METHODS

The study population consisted in 440 men (91 HIV+), attending the Proctology Clinic of Policlinico Umberto I in Rome, for anal diseases. All patients underwent proctological examination and brushing of the anal canal. HPV DNA detection was done by established PCR assays. Nucleotide and amino acid similarity for L1, E6/7, E5 and Long Control Region (LCR) sequences from HIV+ and HIV- patients positive to HPV6 was also examined.

#### RESULTS

About 65% of the anal brushings were positive for HPV-DNA; HPV positivity rate was significantly higher in the HIV+ group (77%) compared to the HIV- (63%) ( $p=0.008$ ). Genotype distribution was similar between the two groups. The most common genotypes in both groups were the LR HPV6 and 11, and the HR genotype HPV16.

115 patients had more than one HPV-DNA test in follow-up visits: HIV+ patients presented a trend toward more reinfections and persistent infections.

Nucleotide polymorphisms were found in the region coding for the capsidic protein L1, for the oncogenic proteins E6, E7 and E5; some of them gave rise to amino acid substitution. Moreover, nucleotide substitutions and deletions were found in LCR. Many of them were conserved only in HIV+ samples.

#### CONCLUSIONS

HPV infection appears to be frequent in both HIV+ and HIV- men. LR genotypes, usually associated to condyloma and low-grade dysplasia, were the most common with the higher persistence length. The persistence of these genotypes in the anal canal, especially observed in HIV+ patients, and the observed mutations could increase their oncogenic risk. Further follow-up of these patients would help to discriminate whether these highly variable sites could play a potential pathogenic role.

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# COMPARATIVE EVALUATION OF THE HUMAN PAPILLOMAVIRUS TYPE 16 L1 PROTEIN EXPRESSED IN PLASMID AND BACULOVIRUS BASED SYSTEMS IN INSECT CELLS AND THEIR APPLICATION IN SEROLOGICAL TESTS

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## BACKGROUND-AIM

Human papillomaviruses cannot be propagated in conventional cell-cultures or have poor yield in culture. Representation of the major antigenic determinants of papillomaviruses (L1 protein), using recombinant molecular biological techniques provide a better approach to the development of sensitive and specific serological assays.

## METHODS

The expression of papillomavirus type 16 L1 in insect cells was investigated using a plasmid (InsectDirect) and a baculovirus system (BacMagic).

## RESULTS

The yields of purified L1 protein from the plasmid and baculovirus systems (10 ml culture; 107 cells) ranged from 455µg/ml to 495µg/ml and from 1.44mg/ml to 1.60mg/ml, respectively. The reliability of the purified L1 to serve as antigens in serological tests was confirmed by the establishment of an ELISA assay. Serum samples previously examined in a seroprevalence study were utilised. The median OD values obtained in plasmid derived L1 based ELISA assays was lower than those found in baculovirus derived L1 ELISA assays. However, reasonable correlation between the baculovirus and plasmid derived L1 based ELISA assay antibody titres was found ( $r=0.75/p<0.004$ ).

## CONCLUSIONS

Expression of the protein under the control of baculovirus P10 very late promoter are similar to the protein expressed under control of plasmid1e1 promoter in the plasmid system. The InsectDirect system appears to be useful for rapid screening of L1 protein expression in insect cells but the BacMagic system is more efficient for the production of recombinant L1 protein.

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# HUMAN HERPES VIRUSES AND RISK OF BLADDER CANCER

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## BACKGROUND-AIM

The incidence of bladder cancer is increasing, and detection of possible risk factors including viral infections in bladder cancer pathogenesis seems necessary to control and prevent the occurrence of bladder carcinoma. The present study aimed to investigate the prevalence and epidemiology of human herpes viruses among patients with bladder cancer in South of Iran.

## METHODS

This descriptive cross-sectional study was supported by research grants of Bushehr University of Medical Sciences (grant number 8037). In this study, we evaluated the presence of HSV-1, HSV-2, CMV, EBV and HHV8 sequences in paraffin-embedded bladder specimens of 181 patients (138 males and 43 females) with different stages and grades of bladder cancer applied to the Shohadaie Khalij-Fars Hospital between 2010–2016. The age range of patients was between 27 to 92 years (mean age  $\pm$  SD:  $64.52 \pm 13.78$  years). Thirty non-cancerous bladder specimens, including 19 normal autopsy specimens and 11 inflamed or fibrotic biopsy specimens, were used as control group. Following deparaffinization of paraffin-embedded tissue sections and extraction of nucleic acid, all samples were tested for the presence of human herpes viruses DNA by nested-PCR using specific primers. This study was approved by the Ethical Committee of Bushehr University of Medical Sciences with reference number B-93-16-19.

## RESULTS

Of the 181 cancerous samples, 34 (18.8%), 5 (2.8%) and 7 (3.9%) were positive for HSV, CMV and EBV, respectively. In control group, HSV and CMV sequences were detected in 2 (6.6%) and 1 (3.3%) of non-cancerous specimens, respectively. HSV showed a higher prevalence in cancerous specimens compared to non-cancerous specimens ( $p=0.048$ ), while EBV was only detected in cancerous specimens. All cancerous and non-cancerous specimens were found to be negative for the presence of HHV8 DNA.

## CONCLUSIONS

The higher prevalence of HSV and EBV in bladder cancer suggests the possible role of these human herpes viruses in the development of bladder cancer in the Iranian population. However, more studies are required to confirm this issue. In addition, no significant statistically association was found between age, gender, stage, and grade of tumor and the presence of human herpes viruses ( $P \geq 0.05$ ).

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### ASSOCIATION OF P16 (CDKN2A) POLYMORPHISMS WITH THE DEVELOPMENT OF HPV16-RELATED PRECANCEROUS LESIONS AND CERVICAL CANCER IN THE GREEK POPULATION

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#### BACKGROUND-AIM

The tumor suppressor protein p16 plays a fundamental role in cell cycle regulation and exerts a protective effect against tumor growth. Two different polymorphisms at positions 540 and 580 at the 3'UTR of exon 3 of p16 gene are implicated in several types of cancer, while their role in cervical cancer development remains rather vague. In the present study we investigated for the impact of p16 genotypes/haplotypes on patients' vulnerability to HPV16-associated cervical disease and examined whether these factors can be used as progression markers in the Greek population.

#### METHODS

A total of 96 HPV16 positive samples and histologically confirmed as LSIL, HSIL and cervical cancer cases along with 33 control cases with normal cervical cytology were tested. DNA was extracted from ThinPrep samples using the chaotropic agent guanidine thiocyanate and the identification of HPV16 DNA was performed through Multiplex PCR using L1 type-specific primer sets. The identification of p16 polymorphisms was performed by PCR-RFLP methodology. The haplotypes were assigned using the online software platform SHEsis (<http://www.analysis.bio-x.cn>), while the association between p16 genotypes/haplotypes and the risk of cervical disease was expressed as the odds ratio (OR) with 95% confidence interval (CI) and their statistical significance was determined by the chi-square test (X2 test).

#### RESULTS

The present analysis revealed that women with p16 540 CG/GG genotype are at a 2.82-fold higher risk of developing HPV16-associated HSIL (OR = 2.82, 95% CI: 1.01-7.87, P=0.04). The G allele can be regarded as a risk factor of developing HSIL in the Greek population (OR = 2.8, 95% CI: 1.1-7.05, P=0.02). Moreover, p16 polymorphism C580T is not associated with the growth of cervical lesion in Greek patients, while 540G/580C haplotype can be regarded as a risk haplotype of developing HSIL (OR = 3.1, 95% CI: 1.2-7.9, P=0.013).

#### CONCLUSIONS

The Greek patients with p16 G (540 CG/GG) genotype display an increased risk of developing HSIL, while individuals with p16 540G/580C haplotype are at considerable risk of growing more severe lesions. The C540G polymorphism has the potential to emerge as a valuable biomarker for HPV16-associated HSIL development in the Greek population.

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### PREVALENCE OF HUMAN PAPILLOMAVIRUS TYPE 16 AND 18 ANTIBODIES (IGG) AMONG GYNAECOLOGICAL PATIENTS AND HEALTH CARE WORKERS

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#### BACKGROUND-AIM

Participation rate of adult women and men in different preventive health care screening control relatively low in our country. 300-500 women die per year from the consequences of human papillomavirus (HPV) infections. The HPV vaccine is not obligatory but complimentary among 13-14 years age groups. In spite of the fact the parents do not prefer the free vaccination.

#### METHODS

For determination of HPV type 16 and 18 antibodies ELISA kits (CUSABIO Biotech CO., LTD. Wuhan, China) were used. Both kits detected HPV 16 and 18 specific IgG antibodies without cross-reactivity or interference. The kits were developed for the detection of naturally acquired anti-HPV 16 and HPV 18 serum antibodies against L1 capsid protein.

Patients; women, who were seen at the outpatient care unit of the Department of Obstetrics and Gynaecology (N=48), University of Szeged, health care workers (HCW) of two laboratories (N=13 and N=49) and nurses of a surgery department (N=75), all working at the University of Szeged. Women who participated in this study did not get HPV vaccine.

#### RESULTS

Averages of age in the four groups were between 30.9-43.2 years. The total number of participants, gynaecological patients and HCWs were 185. The average of age was near ten years lower in the 1st group (gynaecological patients, Outpatient clinics), comparing to the HCWs.

The highest HPV 16 prevalence (N 38, 79.17%) was in the samples of gynaecological patients (1st group: Outpatient clinics), and in the samples of laboratory assistants "2" (3rd group) (N 25, 51.02%). Both HPV 16 and 18 simultaneously (N 18, 24.00%) and HPV 18 alone (N 14, 18.67%) were detected in the samples of registered nurses (4th group). The lowest prevalence of HPV 16, and 18 simultaneously (N 3, 23.08%) was detected in the samples of laboratory assistants "1" (2nd group).

#### CONCLUSIONS

HPV antibodies are the markers of prior exposure to the HPV. The detection of antibodies in serum can be used to study the epidemiology of HPV. In our study the women with positive HPV serological results decided to participate cytological and gynaecological screening. Educating and informing the youth, teenagers and adults are essential at present and in the future.

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### COINFECTION EBV AND HPV IN OROPHARYNGEAL, LARYNGEAL AND ORAL CAVITY CANCER

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#### BACKGROUND-AIM

The Epstein-Barr virus (EBV) is a ubiquitous gammaherpesvirus that infect more than 90% of global population. For the past two decades, increasing interest has been focused on the EBV-associated cancers including Burkitt's lymphoma (BL), Hodgkin lymphoma (HL), nasopharyngeal carcinoma and gastric cancer. Human papillomaviruses are DNA viruses that infect basal epithelial (skin or mucosal) cells. HPV is recognized cause of cervical and head and neck cancers.

#### METHODS

The present study comprised of a group of 146 patients with diagnosed cancer of the pharynx, larynx and oral cavity. The material consisted of the sera and frozen tissue fragments. DNA detection of EBV virus was performed by nested PCR reactions with two pairs of specific primers. HPV detection and genotyping was performed using the INNO-LiPA HPV Genotyping Extra assay, that based on the amplification of 65bp fragment from L1 region of the HPV genome. Statistical analysis was performed to investigate the relationship between EBV and HPV by means of Pearson's and chi-square test with Fisher's exact for small groups. Statistical significance was defined as  $p < 0.05$ .

#### RESULTS

In the study group, the EBV was detected in 63 samples and HPV was detected in 32 samples. Coinfection EBV and BKV was detected in 24 samples. The results of alcohol consumption were statistically significant, 22 patients of coinfection group were drinkers. There were also useful data on histological grading of cancer, 13 patients were in G3 stage, what was the dominant value. Most patient in coinfection group of study – 15 patients, were in N1 stage of cancer.

#### CONCLUSIONS

Both EBV and HPV are confirmed as oncogenic viruses, and have an influence on the development of oropharyngeal, laryngeal and oral cavity cancer. Studies show a significant correlation between alcohol consumption and co-infection with these viruses. In addition, over half the cases of coinfection have been detected in patients with poorly differentiated malignancies – G3 stage, which is a weak prognostic factor.

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### NOVEL ROLE OF HUMAN CYTOMEGALOVIRUS DEUBIQUITINASE IN ONCOGENESIS

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#### BACKGROUND-AIM

Cancer is a multifactorial disease and virus-mediated carcinogenesis is one of the crucial factors, which is poorly understood. Viruses of both genome, DNA and RNA, are capable of causing cancer, wherein DNA tumor viruses have been extensively studied. Human cytomegalovirus (HCMV) is also found to be associated with many cancer types, since last two decades. However, a role of HCMV in cancer remains unknown. Additionally, HCMV infection inhibits synthesis of type I interferons (IFNs) and reduces host immunity. Here, we have investigated the role of HCMV in oncogenesis via inhibiting innate immunity.

#### METHODS

Deubiquitinase (DUB) sufficient- and deficient-HCMV were used to infect mammalian cell and induction of oncogenic properties and inhibition of type I IFNs synthesis were analyzed upon infection. Results were further confirmed by cloning the WT- and Mutant-DUB domain of HCMV-UL48 gene in mammalian expression vector and replicating cancer related experiments in mammalian cell lines.

#### RESULTS

HCMV-deubiquitinase (DUB) enhanced cellular metabolic activity through up-regulation of several anti-apoptotic genes and down-regulation of pro-apoptotic genes expression upon infection. HCMV-DUB also induced several other properties of cancer, such as deregulated cell-cycle, resistant to apoptosis, enhanced invasion & migration, in HCMV infected or UL48 transfected mammalian cells. HCMV-DUB also inhibited synthesis of pattern recognition receptor (PRR)-mediated type I IFNs by deubiquitinating several key signaling molecules in the pathway.

#### CONCLUSIONS

HCMV-deubiquitinase induces carcinogenic properties through inhibiting PRR-mediated type I IFNs via deubiquitination of important signaling molecules. Taken together, our results suggest that HCMV infection may promote oncogenesis by inhibiting innate immunity of the host.

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# PREVALENCE OF HUMAN POLYOMAVIRUSES IN PATIENTS WITH BLADDER CANCER: A PRELIMINARY STUDY

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## BACKGROUND-AIM

The possible role of polyomaviruses in the development of different human tumors lead several researchers to evaluate its prevalence in patients with bladder cancer, though, to date no data have been provided for this group of patients in South of Iran, where has one of the highest numbers of bladder carcinoma in the country. Therefore, this study, funded by Bushehr University of Medical Sciences with grant number 8038, was designed to determine if BK and JC viruses could be detected in bladder cancer tissues.

## METHODS

Between 2010-2016, paraffin-embedded bladder cancer specimens were obtained from 181 patients with bladder cancer (138 males and 43 females), ages ranging from 27 to 92 years. In addition, 19 normal and 11 inflamed or fibrotic tissue samples of 30 non-cancerous patients (16 males and 14 females), ages ranging from 22 to 84 years, were enrolled as controls. Following deparaffinization and extraction of nucleic acid, all samples were used for the detection of BK and JC viruses DNA by nested-PCR targeting large T antigen region of polyomaviruses genome and RFLP. PCR-RFLP products were confirmed by sequencing. This descriptive cross-sectional study was approved by the Ethical Committee of Bushehr University of Medical Sciences with reference number B-93-16-18.

## RESULTS

BK virus sequences were detected in 3 (1.65%) cancerous tissue samples and in 1 (3.3%) normal tissue sample. JC virus sequences were found in 11 (6.07%) cancerous samples and in 1 (3.3%) fibrotic tissue sample. Co-infection with BK and JC viruses was detected in 1.1% of cancerous samples. No significant statistically association was found between age, gender, stage, and grade of tumor and the presence of polyomaviruses ( $P \geq 0.05$ ).

## CONCLUSIONS

Despite the higher prevalence of human polyomaviruses in cancerous specimens (15/181, 8.3%) compared to non-cancerous specimens (2/30, 6.6%), the possible role of polyomaviruses in the onset/progression of bladder cancer cannot be concluded. It seems that air pollution, high-risk occupations, smoking and other risk factor have a more important role in the development of bladder cancer than BK and JC viruses. Nevertheless, the observed predominance of polyomaviruses in cases over controls has to be further explored.

Key words: Bladder Cancer, BK virus, JC virus, Prevalence, Iran

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# EVALUATION OF A SEQUENCE-BASED METHOD FOR HEPATITIS C GENOTYPING (DEEPCHEK® SINGLEROUND PCR AND NS5B/5'UTR SEQUENCING) AND COMPARISON WITH VERSANT HCV GENOTYPE 2.0 ASSAY (INNO LiPA)

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## BACKGROUND-AIM

Adequate hepatitis C virus (HCV) genotyping and subtyping is important for choosing the optimal antiviral treatment regime and the duration of treatment. The VERSANT HCV genotype 2.0 assay (InnoLiPA, Siemens) is frequently used for genotyping of HCV. Sequencing of part of HCV genome can provide more detailed information on HCV genotype (gt), subtype and genetic variation. We compared two genotyping methods: the Deepchek® for sequencing of the NS5B and 5'UTR region (ABL, Luxembourg) and the InnoLiPA.

## METHODS

162 plasma samples from patients with different HCV genotypes, previously tested with InnoLiPA, were selected and genotyped with Deepchek®. Both assays were performed according to manufacturer's instructions.

## RESULTS

Of the 162 plasma samples, 73 samples were gt 1, 22 were gt 2, 39 were gt 3, 22 were gt 4, 2 were gt 5, one was gt 6 and 3 samples were untypable with InnoLiPA. With Deepchek, in all samples a test result for genotyping was obtained. In 134 of the samples genotyping was determined on NS5B, in 28 samples on 5'UTR. Sequences obtained from NS5B were 380-400 nt and from 5'UTR were 210-230 nt. The overall agreement on the genotype level between the InnoLiPA and the Deepchek sequencing method was 95.7%. Seven samples showed discordant genotyping results, of whom 2 samples were 2k/1b recombinant samples (gt 2 on 5'UTR, gt 1b on NS5B).

Discrepancy analysis of the other five discordant results by sequencing the core-part of the genome showed that core-genotype agreed with InnoLiPA in one case and with sequencing of NS5B or 5'UTR in the other cases. The 3 untypable samples with InnoLiPA were all gt 3 by sequencing (2x 3h, 1x 3b).

## CONCLUSIONS

In all 162 samples a genotyping test result was obtained with HCV Deepchek for genotyping.

HCV genotyping by sequencing with Deepchek® agreed in >95% with genotyping with InnoLiPA. Discrepancy analysis showed that two discordant results were due to recombinant strains (1b/2k). In four of the five other discordancies the core genotype correlated with the genotype of NS5B and 5'UTR by sequencing.

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# THE ROCHE COBAS® 6000 ANALYSER AND ROCHE COBAS® 6800 ANALYSER, HEPATITIS C DIAGNOSIS FROM DRIED BLOOD SPOT SAMPLES

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## BACKGROUND-AIM

The use of Dried Blood Spot (DBS) samples has led to an increase in the diagnosis of Hepatitis C virus (HCV) in injecting drug users in the United Kingdom<sup>1</sup>. They are easy and safe to take, with both Anti-HCV and HCV RNA detection possible, enabling identification of actively infected HCV cases from a single DBS sample. To achieve and sustain this level of testing requires the use of automated high throughput analysers. The Roche Cobas® 6000 serological analyser and Cobas 6800® molecular analyser are two such platforms, providing accurate diagnosis for HCV from serum/plasma samples, but these are not currently validated for use with DBS samples. In this study we assess the performance of these analysers for use with DBS samples.

## METHODS

Matching DBS and plasma samples were used for the validation, with the plasma samples acting as the reference result. The validation determined the sensitivity/specificity of the Cobas® Anti-HCV II assay and Cobas® HCV RNA assay from DBS samples, using the Roche Cobas® 6000 and Cobas® 6800 analysers. Final validation results were compared to previous results for Anti-HCV detection from DBS samples using the Abbott Architect, and HCV RNA detection using a laboratory developed NucliSENS® EasyMAg®/Taqman™ based RT-PCR assay.

## RESULTS

Diagnostic sensitivity/sensitivity for Anti-HCV detection from DBS samples using the Cobas® 6000 was 98.1%/100% respectively. Using ROC analysis to set a DBS specific cut-off value improved the sensitivity to 100% while maintaining a specificity of 100%. This compared favourably to the 98.6% sensitive/100% specific results obtained with the Abbott Architect.

Diagnostic sensitivity/specificity for HCV RNA detection from DBS samples using the Cobas® 6800 analyser was 79.1%/100% respectively. The LOD for HCV RNA detection from DBS was 1200IU/ml, significantly less sensitive than the LOD of 373IU/ml achieved using the laboratory developed combination of NucliSENS® EasyMAg® and Taqman based RT-PCR.

## CONCLUSIONS

The performance of the Cobas® 6000, matched with its automation and high throughput, makes it the ideal analyser for identifying Anti-HCV positive patients from DBS samples. Performance of the Cobas® 6800 for HCV RNA detection from DBS samples could not match that of the laboratory developed assay.

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# HBSAG AND HBcrAg QUANTIFICATION IN A FRENCH COHORT OF HEPATITIS B INACTIVE CARRIERS

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## BACKGROUND-AIM

Combinations of markers are used to identify the different phases of chronic Hepatitis B (HBV) infection. According to recent guidelines, a follow-up of 1 year is required to distinguish HBeAg-negative hepatitis from the inactive carrier state (IC) defined by constantly low (<20,000 IU / mL) or undetectable HBV DNA levels and normal serum aminotransferases. HBsAg and hepatitis B core-related antigen (qHBsAg and qHBcrAg) quantification may have an added prognostic value by helping to identify patients at risk of complication. The objective of this work was to assess qHBsAg and qHBcrAg at enrollment in a large prospective French cohort of IC patients (PIBAC cohort)

## METHODS

HBsAg and HBcrAg levels were determined on the Cobas 6000 system (Roche Diagnostics) and the Lumipulse G600 II analyzer (Fujirebio Europe), respectively. HBV genotype was determined by phylogenetic analysis of >600 nucleotides encompassing the overlapping P and S genes.

## RESULTS

From September 2014 to June 2016, the PIBAC cohort enrolled 611 IC patients (285 men and 333 women). At enrollment, mean (+/- SD) HBV DNA and HBsAg levels were 2.43 +/-1.09 and 2.84 +/- 1.5 log IU/ml. HBcrAg levels were quantifiable (>3 log), detectable (2-3 log) and undetectable (<2log) in 10.8%, 44.7%, and 44.5%, respectively. Genotyping was available for 73%, with 37.4% E, 26.6% A, 24.3% D, 6.1% B, 5.1% C, and 0.4% F. HBsAg titers did not differ according to genotype but were significantly lower in patients where genotyping was unsuccessful (1.92 +/-2.12 vs. 3.17 +/- 1.04, p<0.0001). Accordingly, we observed a correlation between HBsAg and HBV DNA levels at enrollment. HBsAg levels were higher in patients with detectable or quantifiable HBcrAg: 3.01 +/-1.44 and 3.48 +/-1 vs. 2.48 +/-1.61 log IU/ml, p=0.005. In addition, HBcrAg titers were higher for genotype B and C infected-patients (p=0.01)

## CONCLUSIONS

In this large and genotypically diverse cohort of inactive HBV carriers, significant correlations were observed at enrollment between HBV DNA, HBsAg and HBcrAg levels. A 5 years follow-up is planned and the prognostic value of these additional makers will be prospectively assessed. We also observed a genotype effect on HBcrAg quantification that needs further investigations.

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# PERFORMANCE EVALUATION OF NEW AUTOMATED IMMUNOASSAY TESTS, VIDAS® ANTI-HEV IGM AND IGG ASSAYS, IN EUROPEAN AND NON-EUROPEAN POPULATIONS

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## BACKGROUND-AIM

High performance anti-hepatitis E virus (HEV) serologic assays are crucial for diagnostic and epidemiology of infection. Our objective was to evaluate the performance of the new bioMérieux diagnostic tests, VIDAS® Anti-HEV IgM, As HEV circulates as 4 different genotypes, both VIDAS® Anti-HEV assays were evaluated for the detection of antibodies against various HEV genotypes in samples from European and non-European patients.

## METHODS

The 2 VIDAS® Anti-HEV IgM and IgG prototypes were developed using antigen corresponding to HEV ORF2 and/or ORF3. Sensitivity and specificity of each assay was determined by testing European samples with HEV infection characterized by HEV RNA PCR and compared with. Wantai IgM and IgG tests. About 100 samples from both European immunocompetent and immunocompromised patients were tested with both prototypes. Cross reactivity were assessed using samples that tested positive for hepatitis A, B or C, dengue, malaria, CMV, EBV or rheumatoid factor. 100 samples from China and 1000 from Burkina Faso, were tested as well with VIDAS® Anti-HEV IgM and IgG assays.

## RESULTS

For Anti-HEV IgM assay, the sensitivities in immunocompetent patients were: 94.7% (ORF2 prototype), 96.5 % (ORF2/3 prototype), and 96.5% (Wantai); in immunocompromised patients they were: 76.1% (ORF2 and ORF2/3 prototypes), and 78.3% (Wantai). Specificity was 95.8 / 96.2% (ORF2 prototype) and 97.9 / 98.1 % (ORF2/3 prototype) for immunocompetent/immunocompromised patients. Results for the 2 VIDAS® Anti-HEV IgG prototypes were previously published and demonstrated an excellent performance for both prototypes (Abravanel, et al, J Clin Virol 2017). Limited cross-reactivity towards related pathogens were demonstrated for both VIDAS® Anti-HEV IgM and IgG assays. Sensitivity study performed with non-European samples, demonstrated that VIDAS® Anti-HEV IgM and IgG are suitable for the efficient antibody detection against different HEV genotypes.

## CONCLUSIONS

Sensitivity and specificity VIDAS® Anti-HEV IgM and IgG are comparable to CE-marked tests and suitable for the detection of antibodies anti-HEV in European and non-European populations.

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# NAIL SCISSORS AND FINGERNAILS AS RESERVOIRS OF HEPATITIS B VIRUS DNA: ROLE OF NAIL SCISSORS IN HOUSEHOLD TRANSMISSION OF HEPATITIS B VIRUS

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## BACKGROUND-AIM

No study in current literature has scientifically clarified the transmission of HBV via personal hygiene tools, including nail scissors. In this study, we determined risk of household transmission of HBV via nail scissors. Moreover, we assessed whether nails from HBV-infected people contained HBV, and evaluated correlations of HBV DNA levels of serum, nail specimens, and nail scissors.

## METHODS

HBsAg-positive seventy patients (63 positive for HBV DNA) treated for HBV infection and 27 healthy individuals (control group) were included in this study. Nail scissors and nail specimens were requested from the subjects. Real-time PCR method was used to detect HBV DNA levels in sera, nail and nail scissors.

## RESULTS

HBV DNA was detected on 27% nail scissors and in 50% of nail specimens from 63 serum HBV-positive patients. According to ROC analysis, a serum HBV DNA level of >105 IU/ml was associated with 71.8% probability of nail scissors being HBV DNA-positive (p<0.05). Also, a serum HBV DNA level of >107 IU/ml was associated with 71.5% probability of fingernails being HBV DNA-positive (p>0.05). Shared-use of nail scissors was correlated with HBsAg positivity and anti-HBc total/IgG positivity (r=0.284, p=0.017, r=0.325, p=0.006, respectively). HBV is present at low levels in the nails of persons with high serum HBV DNA levels. A high HBV DNA level was detected in nail scissors used by serum HBV DNA-positive patients.

## CONCLUSIONS

According to our results, HBV is present at low levels in the nails of persons with high serum HBV DNA levels. A high HBV DNA level was detected in nail scissors used by serum HBV DNA-positive patients. Nail scissors, shared use of nail scissors, and the duration of shared use play important roles in household transmission of HBV. Members of a household with an HBV-infected individual should not use shared tools such as nail scissors, which may come into contact with blood and body fluids. The level of education within society in this regard should be increased via the mass media.

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**VALIDATION OF THE DIASORIN LIAISON XL HBsAg QUANT CLIA FOR USE WITH CADAVER PLASMA**S. Best<sup>1</sup>, L. Arvanitis<sup>1</sup>, S. Jalota<sup>2</sup><sup>1</sup>National Serology Reference Laboratory (NRL), Melbourne, Australia<sup>2</sup>School of Science, RMIT University, Melbourne, Australia**BACKGROUND-AIM**

The National Serology Reference Laboratory, Australia (NRL) wished to use the DiaSorin Liaison XL Murex HBsAg Quant CLIA (XL HBsAg CLIA) to screen for HBsAg in serum/plasma from deceased tissue donors. However, cadaver serum/plasma is not a validated sample type for use in the XL HBsAg CLIA according to the instructions for use (IFU). Australia's regulatory body requires that any deviation from the manufacturer's IFU must be validated and included on the Australian Register of Therapeutic Goods as an in-house in vitro diagnostic device.

**Aim**

To validate the use of cadaver plasma with the XL HBsAg CLIA.

**METHODS**

Forty two cadaver and 42 pre-mortem plasma specimens negative for HBsAg and anti-HBs were retrieved from NRL's specimen archive. Of the cadaver specimens, 28 were collected within 24 hours of death, 13 > 24 hours and one was unknown. All specimens were from different individuals. Each specimen was divided into three aliquots, one for spiking at low HBsAg concentration, another at medium concentration and the last to remain unspiked. Five different HBsAg positive specimens were used for spiking, each used to spike up to nine cadaver and nine pre-mortem aliquots at both HBsAg concentrations. All aliquots were tested on the XL HBsAg CLIA. Results from cadaver and pre-mortem aliquots that had been spiked with the same HBsAg positive specimen were compared. Results were required to fall within 25% of each other to be accepted. Further, all spiked aliquots were required to be positive and all unspiked aliquots negative on the XL HBsAg CLIA. Precision was assessed by testing one cadaver and one pre-mortem low HBsAg spiked specimen 20 times over 2 days. Coefficients of variation (CV) were required to be <15%.

**RESULTS**

All spiked and non-spiked cadaver and pre-mortem aliquots gave the expected results on XL HBsAg CLIA. Comparing the 10 mean HBsAg results from cadaver and pre-mortem aliquots spiked with one of five HBsAg positive specimens at two concentrations, four were 4-14% lower and the remaining six were 6-16% higher. The CVs in the cadaver and pre-mortem precision specimens were 4.4% and 5.1% respectively.

**CONCLUSIONS**

Results on the XL HBsAg CLIA for cadaver specimens met NRL's acceptance criteria, showing that cadaver plasma is a valid specimen type to use with the XL HBsAg CLIA.

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**PERFORMANCE EVALUATION OF THE APTIMA® HCV QUANT DX ASSAY ON THE FULLY AUTOMATED PANTHER SYSTEM IN COMPARISON TO COBAS® HCV TEST FOR COBAS® 6800/8800**A. Ebel<sup>1</sup>, C. Bali<sup>1</sup>, L. Guis<sup>1</sup>, S. Merlin<sup>1</sup><sup>1</sup>Eurofins Biomnis Laboratory-Ivry Sur Seine-France**BACKGROUND-AIM**

Quantification of HCV RNA load plays a key role in management of HCV infected patients, before and during antiviral therapy.

In this study we compared the overall performance of the Hologic Aptima® HCV Quant Dx (Hologic), a quantitative HCV assay based on real-time Transcription Mediated Amplification (TMA) technology, developed for the Panther system, with the Roche cobas HCV assay for c6800/8800.

**METHODS**

276 serum samples (84 fresh and 192 frozen) from HCV-infected patients and viral load results based on c6800/8800 were retested with Aptima.

In addition 110 retrospective samples from HCV-infected patients with a defined genotype (GT), (GT1a:19, GT1b:21, GT2:19, GT:18, GT4:19, GT5:14) were tested.

Serial dilutions of reference panels (Acrometrix) or clinical samples (GT1a and GT4) were used to assess repeatability and reproducibility.

Risk of contamination was evaluated by testing 41 consecutive high positive and negative samples.

**RESULTS**

Deming regression showed very high concordance between the assays, for prospective and retrospective samples ( $y = 1.02x - 0.29$ ,  $R = 0.99$ ,  $y = 1.05x - 0.41$ ,  $R = 0.9839$ , percentage of agreement 92.9 and 85.4%, respectively).

Discrepant results were observed only around the threshold values.

For all genotypes tested (GT 1 to 5) excellent correlation between the 2 assays was observed.

Mean difference between measured and expected values was < 0.42 Log for serial dilution using Acrometrix HCV panel (7.7 to 2.0 Log), N=4 per dilution level. Repeatability was excellent with SD ranging from 0.11 to 0.03 Log. Reproducibility assessed by testing serial dilutions (3 to 1.4 Log) of serum samples from patients (GT1a and GT4), N= 10 per dilution level, was excellent for both genotypes, (SD's ranging from 0.05 to 0.15 Log).

No cross contamination was observed.

**CONCLUSIONS**

The Hologic Aptima® HCV Quant Dx assay on the fully automated Panther system gave highly comparable performance to the cobas® 6800/8800 system for clinical samples. Reproducibility and repeatability using dilution series were excellent. The Aptima assay, with just 0.5 ml sample input volume, was easy to use and could generate 120 test results in less than four hours. Overall the Hologic Aptima® assay is highly suitable for use in the clinical laboratory setting.

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### 5' NCR AND NS5B REGIONS FOR HEPATITIS C VIRUS GENOTYPING – RESULTS FROM WESTERN PART OF TURKEY

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#### BACKGROUND-AIM

Genotyping of hepatitis C virus (HCV) has become useful for clinical decision making and phylogenetic analysis is useful in epidemiological studies. Because of the high variability of HCV, it is important to target genome regions with high conservation for the sensitivity, but considerable variability between genotypes. Reliable genotyping assays are needed to be evaluated, since subtyping has recently become important for treatment regimens with direct acting antivirals. It is aimed to compare 5' non-coding region (5'NCR) and non-structural 5B (NS5B) of HCV genome for effective genotyping.

#### METHODS

Nucleic acid sequences from 5'NCR and NS5B regions were analysed from fifty plasma samples of chronically HCV infected patients sent to the Clinical Virology Laboratory of Ege University Medical Faculty, Medical Microbiology Department for genotyping. RNA samples isolated with EZ-1 mini viral kit (Qiagen) were reverse transcribed to cDNA and amplified with region specific primers (nested PCR for 5'NCR and semi-nested PCR for NS5B) and then nucleotide sequences (8256-8636 for NS5B and -279 to -29 for 5'NCR) were determined by using Big Dye Terminator kit with ABI Prism 310 DNA sequencer (PE Applied Biosystems). The genotypes were determined by comparing its sequence with those of HCV prototypes obtained from GenBank (NCBI Blastn and Viral genotyping tool), aligned (DNASTar MegAlign) and DNAdist program.

Acknowledgements: Yaşar Gökçe for his technical support.

#### RESULTS

Genotype determination could be done both with 5'NCR and NS5B analysis. Results from both regions were always concordant for the genotype. The NS5B analysis permitted the identification of the subtype in all samples, whereas 5'NCR analysis did not in 60%. 35 isolates were typed as genotype (GT) 1B, two as GT1a, seven as GT4, three as GT3, two as GT5, and one as GT2.

#### CONCLUSIONS

Analysis of both 5'NCR and NS5B regions are reliable and convenient for HCV genotyping, but NS5B region analysis is needed for subtyping GT1.

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### HEPATITIS B VIRUS GENOTYPES IN BLOOD DONORS IN SLOVENIA

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#### BACKGROUND-AIM

Hepatitis B virus (HBV) infection is associated with a wide spectrum of clinical manifestations, ranging from acute to chronic hepatitis, cirrhosis and hepatocellular carcinoma. The genome of HBV has been classified into eight genotypes (A to H) which have been found to influence the chronicity rate, the course of disease and the response to treatment. Our goal was to determine the distribution of HBV genotypes in the population of HBV infected blood donors in Slovenia.

#### METHODS

78 plasma samples, collected from the HBV infected blood donors from 2000 to 2015 were included in the study. Before genotyping, all plasma samples were stored at -25°C. Viral DNA was extracted from plasma samples, using EZ1 Virus Mini Kit v2.0 (Qiagen, Germany). A real-time genotyping method has been developed and optimized, using TaqMan chemistry on ABI PRISM Sequence Detection System 7900HT (Applied Biosystems, UK).

#### RESULTS

Genotype D was detected in 80.8% (63/78) of the analysed plasma samples, while genotypes A and B accounted for 7.7% (6/78) and 1.3% (1/78) of tested samples respectively. The remaining 3.8% (3/78) of blood donors were infected with mixed genotypes, i.e., A/D and A/C. It was not possible to determine the genotype of HBV in 6.4% (5/78) of blood samples.

#### CONCLUSIONS

This is the first study of the distribution of HBV genotypes in HBV infected blood donors in Slovenia. Our results correlate to the Slovenian geographic location, where a predominant infection with genotype D and only minor to moderate infection with genotype A are expected. Surprisingly, one genotype B and one C were detected, which are common in the Pacific region. In samples where HBV genotyping was unsuccessful, an additional ultracentrifugation step or a modified genotyping assay should be applied.

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### CURRENT HEPATITIS A VIRUS (HAV) OUTBREAK: A SINGLE CENTRE EXPERIENCE IN NORTH LONDON, UNITED KINGDOM

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#### BACKGROUND-AIM

Hepatitis A virus (HAV) causes an acute, self-limiting infection. Transmission is usually by faecal-oral route but sexual transmission has been associated with a current European outbreak among men who have sex with men (MSM). Public Health England (PHE) describes an outbreak of HAV genotype 1a strains, previously not seen in England. At least 74% of these cases were among MSM, and 63% were in London (Public Health England Weekly Report 2017).

#### METHODS

We describe a case series of acute Hepatitis A infection observed at a large teaching hospital in North London between January – June 2017.

#### RESULTS

We identified 11 cases of acute Hepatitis A, defined by the presence of HAV IgM antibodies. All but one patient had detectable HAV IgG antibodies at the time of presentation. The patients had a median age of 36 years (range: 24-59 years) and were predominantly male (10 male, 1 female). All patients had an identifiable risk factor including some with a recent travel to a known area of outbreak. Five (50%) patients were MSM, all of whom reported recent unprotected sexual exposure. Eight (80%) patients reported recent travel in Europe, of which 6 (75%) had recently returned from Italy and/or Spain. HAV RNA was detected in 8 samples and all of these patients were genotype 1a: 12.5% were strain 1, 37.5% strain 2 and 50% strain 3 (2 further results pending). The predominant presenting features of strain 1 and 2 cases were abdominal pain and vomiting, whereas strain 3 cases presented with jaundice and lethargy. Nine patients needed hospital admission for supportive management (80%, median duration of admission = 2 days [range: 1-3]). Transaminases were very high in patients, but were not significantly different between strains (median ALT= 3052 U/L [range: 1064-10516], median AST=1865 U/L [range: 174-5468], median total bilirubin=123 µmol/L [range: 55-236]. No information was available regarding immunisation status of the patients.

#### CONCLUSIONS

Our single centre experience is in keeping with the current UK outbreak with a new HAV genotype. Routine vaccination of at risk population should be implemented to reduce infection and onward transmission.

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### EFFECT OF BASELINE Y93H RAS IN HEPATITIS C GENOTYPE 3 FOR NS5A COMBINATION TREATMENTS: REAL-LIFE EXPERIENCE FROM A MULTICENTER STUDY IN SWEDEN AND NORWAY

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#### BACKGROUND-AIM

The prevalence of HCV baseline NS5A Y93H resistance associated substitution (RAS) is approximately 7% in genotype (GT)-3 of Nordic DAA-treatment naive patients. This baseline RAS may have a negative impact on sustained viral response (SVR) as indicated by the ALLY-3 and ASTRAL 3 studies.

#### METHODS

Consecutive patients from Uppsala, Gävle and Tromsø (intervention group) and from Örebro, Falun, Stockholm and Bodö (control group) were included. In this interim report, patients with GT 3 were included in the study during Q3 2014 – Q1 2017.

A prospective intervention study was performed, where treatment in the intervention group was based on baseline RASs and at the responsible MD's discretion: with Y93H e.g. prolonged daclatasvir plus sofosbuvir treatment was often used (or Eplclusa when available). Recommended treatment, according to the National Boards, was given to patients without baseline RASs (i.e. Y93H) in the intervention group and for all the patients in the control group. Ribavirin was also added at the responsible MD's discretion. The original study is planned for 100 patients in each arm during Q3 2014 – Q3 2017 with a power of 90%.

#### RESULTS

Preliminary results were obtained from a total of 91 patients, including an intervention group of 57, and a control group of 34 patients. The prevalence of Y93H in the intervention group at baseline 5.3% (3/57). The non-SVR rates in the intervention group compared to the control group were 8.77% (5/57) to 11.76% (4/34), respectively. However, the limited number of patients in the control group had lower liver damage and more 24 week treatments than the intervention group. Three patients with pre-existing Y93H in the intervention group (3/57), were cured (SVR) with prolonged, 24 weeks, NS5A-inhibitor plus sofosbuvir treatment without ribavirin, or with Peg-INF/RBV + sofosbuvir.

Additions of ribavirin in both genotype groups indicate a positive effect on SVR12.

#### CONCLUSIONS

The number of patients is too low to draw any final conclusions regarding SVR12, but the preliminary results indicate higher SVR12 for the intervention group. All three patients with pre-existing Y93H were successfully treated in the intervention group.

The study is ongoing and completions of patient data are pending as well as analysis of baseline RAS Y93H on patients from the control group.

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### PERFORMANCE OF THE DXN VERIS HBV AND HCV ASSAYS FOR VIRAL LOAD QUANTIFICATION IN PATIENTS WITH HEPATITIS B OR HEPATITIS C

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#### BACKGROUND-AIM

Viral load monitoring is an essential tool in the management of patients with hepatitis B to evaluate disease status and response to therapy. For HCV infected patients, RNA viral load assessment is recommended prior to treatment and to evaluate response during and after direct-acting antiviral (DAA) therapy. The DxN VERIS Molecular Diagnostics System is a fully-automated, random-access system for the determination of viral load in infected patients. The aim of this study was to assess the performance of the VERIS HBV and HCV assays against the Roche COBAS AmpliPrep / COBAS TaqMan (CAP-CTM) system assays, which are in routine use in our laboratory.

#### METHODS

For method comparison, plasma from 369 HBV infected patients were selected for analysis on both platforms. For HCV, 117 plasma samples, taken at different time points during treatment, from 39 patients undergoing DAA therapy were selected for analysis on both the DxN VERIS and Roche CAP-CTM instruments.

#### RESULTS

For the HBV method comparison, of the 369 specimens tested, 35 samples were "not-detected" on both systems, a further 25 samples were detected but not quantified on both systems. Three samples were quantified on the VERIS assay but were "not detected" on the Roche assay. For 255 samples that were quantified, the correlation coefficient ( $r^2$ ) was determined to be 0.90 with an observed bias of -0.43 log IU/mL.

For the HCV method comparison, of the 117 samples tested, 35 were "not detected" by both assays and a further 5 were detected but not quantified. Three samples were quantified with the VERIS assay but "not-detected" on the Roche assay, and one sample quantified by the Roche assay was "not-detected" using the VERIS assay. For the 60 samples that were quantified, the correlation coefficient ( $r^2$ ) was 0.86 and the observed bias was -0.29 log IU/mL.

#### CONCLUSIONS

Method comparison between the VERIS HBV and Roche HBV assays demonstrated an overall concordance of 80%. Regarding HCV, the overall concordance between both systems was 85%. Patient monitoring profiles illustrate that the VERIS HCV assay is an useful tool in monitoring viral load levels during treatment.

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### MULTIPLE HBV TRANSFUSION TRANSMISSION BY DONOR WITH OCCULT HEPATITIS B (OBI)

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#### BACKGROUND-AIM

OBI was defined as the presence of HBV DNA in the liver (with or without HBV DNA in serum) without HBsAg as determined by using the currently available assays. On the basis of the HBV anti-HBc and/or anti-HBs antibody profile, OBI may be distinguished as seropositive or seronegative. HBsAg screening of blood donors is mandatory in the Czech Republic, unlike NAT or anti-HBc screening which is performed in some blood banks only.

#### METHODS

Primary screening of HBsAg: DiaSorin Liaison XL HBsAg. NAT HBV DNA in Baxalta: cobas® TaqScreen MPX Test. NIPH HBV serology: Architect Abbott tests. HBV DNA: cobas AmpliPrep/cobas TaqMan v.2

#### RESULTS

Hepatitis B was diagnosed in patient S.H. in December, 2016 five months after cardiological operation. Erythrocytes from 10 donors were given to this patient during operation. Testing of archived samples confirmed HBsAg negativity of these 10 donors. Only one of these samples from donor J.P. was reactive for anti-HBc antibody. Retrospective analysis showed that J.P. donated blood in the year 2016 in March, July, and September. Archive samples from this donation were HBsAg negative, anti-HBc reactive, anti-HBs 11 I.U./L and levels of HBV DNA were around 20 I.U./mL. Erythrocytes from these donations were given to other two recipients. One of them died on primary oncological illness before beginning of the investigation of HBV transmission. Another recipient was not HBV infected due to high level of anti-HBs passively transferred by previous plasma donation. Plasma from the March blood donation was given to an oncological patient and HBV was transmitted. Plasma from two other donations were provided to Baxalta for industrial processing. Results of NAT HBV DNA in Baxalta in the pool of 96 samples were negative (June donation) and positive (September donation).

#### CONCLUSIONS

OBI was proved in blood donor J.P. Three recipients of erythrocytes from three blood donations and one of plasma donation of this donor were exposed to HBV. Two of them were probably HBV infected. Transmission of HBV could be prevented, if anti-HBc screening or NAT HBV DNA screening was performed. But, NAT test in this case, when HBV DNA level was around 20 I.U./mL, would be effective and reliable in pool lower than 96 samples.

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### NINE-YEAR DISTRIBUTION PATTERN OF HEPATITIS C VIRUS GENOTYPES IN SOUTHERN ITALY

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#### BACKGROUND-AIM

Different hepatitis C virus (HCV) genotypes exhibit differences in disease pathogenesis and progression, as well as in disease outcomes and response to therapy. Tracking the change of HCV genotypes in various epidemiological settings is critical for both disease surveillance and the development of improved antiviral treatment. Here, we tracked the changes in the prevalence of the distribution of HCV genotypes in Southern Italy in three different periods 2006-2008, 2009-2011 and 2012-2014.

#### METHODS

HCV genotypes distribution was analysed in 535 HCV-RNA positive patients with chronic HCV infection, observed from 2012 to 2014, and compared with our previous study, which recorded data in the period 2006-2008 and 2009-2011. Pearson chi-square test and t-test were used to statistically analyze the results.

#### RESULTS

In all the periods analysed, genotype 1 is predominant, even if its prevalence decreased from 2006 to 2014 (64.3% in 2006-08, 62.4% in 2009-11 and 60.8% in 2012-14). On the contrary, in the same period, the genotype 2 prevalence increased (27.9% in 2006-08, 31.7% in 2009-11 and 35.2% in 2012-14), whereas genotype 3 seems to show a decrease during the time (6.8% in 2006-08, 4.7% in 2009-11 and 3.2% in 2012-14). Subtype 1b, particularly common in females compared to males in the past (64.3% vs 39.3% in 2006-08,  $p < 0.001$  and 54.0% vs 41.5% in 2009-11,  $p < 0.05$ ), seems to be now quite equally distributed between males and females (52.7% vs 56.6%). Instead, the male/female ratio for genotype 3 is highly decreased from 2006 to 2014. The prevalence of patients with genotype 1b in the age range 31-40 years is significantly higher in the 2012-14 period than in both previous periods (53.8% vs. 16.6% in 2009-11,  $p < 0.001$  and 13.4% in 2006-08,  $p < 0.001$ ) and lower in the over-60-year-olds (57.9% vs. 59.6% in 2009-11 ).

#### CONCLUSIONS

Genotype 1b, historically the most prevalent in Southern Italy, is still predominant; however, when comparing the three time periods, genotype 2 seems to show an increase in the general population not related to age or gender, whereas genotype 1b, even if the most common in the elder population, shows a significantly increase in the under 40 years old population

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### EVALUATION OF THE DXN VERIS HBV ASSAY FOR THE QUANTIFICATION OF HBV DNA FROM WHOLE BLOOD AND DRIED BLOOD SPOT

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#### BACKGROUND-AIM

Hepatitis B virus (HBV) infections are highly prevalent in resource-limited settings mostly in Africa and the Pacific. Dried blood spots (DBS) on filter paper have been successfully used to diagnose and monitor several infectious diseases. The aim of this study was to investigate the quantification of HBV viral load (VL) on DBS or unprocessed whole blood (WB) using the new fully automated DxN VERIS system (Beckman Coulter).

#### METHODS

Non infected WB samples were spiked with plasma containing a known concentration of HBV DNA (genotype E). Serial 1:10 dilutions were performed in WB to assess assay linearity. Each DBS was prepared by deposition of 50  $\mu$ L of tainted WB on a 12-mm paper card. Spots were left overnight at room temperature for drying and then frozen at -80°C for a minimum of 12 days. Rigorous decontamination procedures were applied between each DBS puncture and elution of materials from spots was done using phosphate-buffered saline (PBS). DBS, unprocessed WB and plasma prepared from serially diluted spiked WB were analyzed with the DxN Veris system. To measure WB HBV-VL, a 1:10 dilution in PBS was done beforehand. Negative controls (blank spots) were tested between each DBS to assess cross-sample contamination.

#### RESULTS

HBV-VL ranged from 2.30 to 8.21, 2.89 to 7.65 and 2.48 to 7.61 log IU/mL in plasma, WB and DBS, respectively. An excellent correlation was found between plasma and WB measurements ( $R^2=0.99$ ;  $P<0.0001$ ) and also between plasma and DBS ( $R^2=0.99$ ;  $P<0.0001$ ). Bland-Altman analysis revealed a mean difference measurement of 0.08 log IU/mL between WB and plasma and a mean difference measurement of 0.01 log IU/mL between DBS and plasma. Specificity of HBV detection was slightly impaired by possible contamination following DBS puncture despite thorough cleaning during the procedure. Two out of 6 negative controls provided a low positive signal.

#### CONCLUSIONS

Recent improvements of molecular biology automated systems make them attractive, even in not expert settings. This study showed that the DxN VERIS system could accurately quantify HBV-VL from DBS but also directly from WB following a simple dilution. While DBS represent an interesting alternative, extreme care should be taken to avoid cross sample contamination.

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**HBsAg ESCAPE MUTATIONS IN HUNGARY**Z. Rottenberger<sup>1</sup>, F. Bakó<sup>1</sup>, I. Vályi-Nagy<sup>1</sup>, E. Újhelyi<sup>1</sup><sup>1</sup>Department of Molecular Biology, Szent László Hospital, Budapest**BACKGROUND-AIM**

Hepatitis B virus (HBV) infection is still a serious worldwide problem, a leading cause of acute and chronic liver disease including cirrhosis and liver cancer, which ranks as the third cause of cancer deaths worldwide. Vaccination is the most effective strategy for primary prevention of the infection. Hepatitis B is now considered a largely treatable and preventable disease, thanks to the availability of effective antiviral drugs.

Central core of Hepatitis B surface Antigen (HBsAg) is involved in binding to antibodies directed against HBsAg. Anti-HBs (a-HBs) is the main component of vaccine induced immunity. The success of the vaccination programs has been challenged by the discovery of mutant

viruses showing amino acid substitutions in HBsAg MHR, which may lead to evasion of vaccine-induced immunity. However, the emergence of these mutations has not yet raised concern since it was shown that they develop slowly.

The aim of this study was to investigate the prevalence of HBsAg escape mutants by routine ELISA screening tests and by HBV PCR in a group of Hungarian HBV infected patients.

**METHODS**

From 2015 to 2017, HBV positive patients' medical records (HBsAg and anti-HBs serological status and HBV PCR results) were collected and statistically analyzed.

**RESULTS**

Analyses were performed in 766 patients. Persistently positive HBsAg, a-HBs and HBV PCR results were found in 14 cases (age: 27-75 years, median 53; HBsAg positivity: S/Co:26,7-45,8; HBV DNA levels: from 21 IU/ml to 7,91x10<sup>6</sup> IU/ml, median 7,4x10<sup>5</sup>; a-HBs levels: >12 IU/L).

**CONCLUSIONS**

These patients were not vaccinated against HBV, meaning that they are not vaccine induced escape mutations, and a-HBs in these cases has no significant protective role.

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**MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS IN END-STAGE RENAL DISEASE PATIENTS UNDER HEMODIALYSIS**R. Alves Da Silva<sup>2</sup>, J. De Souza Todão<sup>2</sup>, F. Luiz Kamitani<sup>1</sup>, A.E. Benedito Silva<sup>3</sup>, R.J. De Carvalho-Filho<sup>3</sup>, M.L. Cardoso Gomes Ferraz<sup>3</sup>, I.M. Vicente Guedes De Carvalho<sup>2</sup><sup>1</sup>Instituto Butantan<sup>2</sup>Instituto Butantan / Laboratório de Hepatologia Molecular Aplicada (LHeMA) UNIFESP<sup>3</sup>Laboratório de Hepatologia Molecular Aplicada (LHeMA) UNIFESP**BACKGROUND-AIM**

New direct-acting antiviral (DAA) agents are in development or already approved for the treatment of chronic hepatitis C virus (HCV) infection. The effectiveness of these drugs is related to the previous existence of resistant variants. Certain clinical conditions can allow changes in immunological characteristics of the host and even modify genetic features of viral populations. The aim of this study was to perform HCV molecular characterization from samples of end-stage renal disease patients on hemodialysis (ESRD-HD).

**METHODS**

Nested PCR and Sanger sequencing were used to obtain genetic information from the NS5B partial region of a cohort composed by 86 treatment-naïve patients. Genomic sequences from the Los Alamos databank were employed for comparative analysis. Bioinformatics methodologies such as phylogenetic reconstructions, informational entropy and mutation analysis were used to analyze datasets separated by geographical location, HCV genotype and renal function status.

**RESULTS**

ESRD-HD patients presented HCV genotypes 1a (n=18), 1b (n=16), 2a (n=2), 2b (n=2) and 3a (n=4). Control subjects were infected with genotypes 1a (n=11), 1b (n=21), 2b (n=4) and 3a (n=8). Dataset phylogenetic reconstruction separated HCV subtype 1a into two distinct clades. The entropy analysis from the ESRD-HD group revealed two amino acid positions related to an epitope for cytotoxic T lymphocytes and T helper cells. Genotype 1a was found to be more diverse than subtype 1b. Also, genotype 1a ESRD-HD patients had a higher mean of amino acid changes in comparison to control group patients.

**CONCLUSIONS**

The identification of specific mutations on epitopes and high genetic diversity within the NS5B HCV partial protein in hemodialysis patients can relate to host immunological features and geographical distribution patterns. This genetic diversity can affect directly the new DAA's resistance mechanisms.

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**TESTING PERFORMANCE OF XPert HCV VL USING DILUTED SAMPLES**L. Coltella<sup>1</sup>, S. Ranno<sup>1</sup>, G. Pizzichemi<sup>1</sup>, M.D. Caione<sup>1</sup>, C. Concato<sup>1</sup><sup>1</sup>Unit of Microbiology Virology and Parasitology, Laboratory Department, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy**BACKGROUND-AIM**

Collect the correct amount of sample in order to detect the presence of HCV virus could be a critical point in pediatric patients. Xpert HCV Viral Load assay, performed on GeneXpert instrument, requires minimum 1 mL of plasma or serum in order to carry out the test. Xpert HCV VL presents a limit of quantification at 10 IU/ml and a limit of detection at 4 IU/ml. Thus exploiting the sensitivity of Xpert HCV VL we tested the performance of this assay using a lower amount of sample. Particularly, we tested samples with a volume ranging from 200 to 1000 microlitres.

**METHODS**

We analyzed 38 samples of HCV positive patients. Samples have been tested with two different methods used in our laboratory to compare HCV RNA levels: Xpert HCV VL (Cepheid, Sunnyvale, CA) and Abbott m2000 RealTime HCV assay (Abbott Diagnostics, Hoofddorp, the Netherlands). All samples tested, showed a detectable viral load, ranging from 35 to  $2,11 \times 10^7$ . In order to process the Xpert HCV VL test, samples dilutions were carried out using Molecular Grade Water.

**RESULTS**

To compare quantitative results obtained using the two methods, 12 out of 38 samples were tested not diluted and no discrepancy had been detected ( $\pm 0,5$  Log). The remaining 26 samples were diluted with different dilution factor, particularly: 1.33, 2, 4, 5. In order to make a comparison with Abbott, results provided by the GeneXpert system, were calculated considering the dilution factor. Correlation of these results, confirmed no significant differences, always lower than  $\pm 0.9$  log, with the exception of just one sample with a very high load.

**CONCLUSIONS**

The overall results reflect a good level of correlation with Abbott methods even using diluted samples. Dilution does not affect the efficacy of the Xpert HCV VL assay, allowing to perform the test with a good accuracy even when the amount of sample is lower than 1 mL, not infrequent in pediatric patients.

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**ALTERNATIVE EVOLUTIONARY HISTORIES OF THE HEPATITIS B VIRUS AND ITS POTENTIAL IMPACT ON THE CLINICAL OUTCOMES OF THE DISEASE**B.A. Godoy<sup>1</sup>, N.J.R. Fagundes<sup>1</sup><sup>1</sup>Genetics Department, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil**BACKGROUND-AIM**

Hepatitis B Virus (HBV) is a major health problem, being responsible for chronic infection of ~250 million people worldwide. Phylogenetic studies highlight its long-term co-evolution with humans. Progression to severe clinical outcomes are related to both virus and host genetic background. Hence, distinct HBV genotypes may have different clinical outcomes in different host populations. Paraskevis et al. (2015) suggested that the branch leading to genotypes F/H, typical of Native American populations, had an accelerated substitution rate. If this is true, then the natural history of HBV-F/H infection could depend on the ancestry of affected individuals. However, inferences of evolutionary processes are highly dependent of the specific phylogenetic story assumed. The goal of this study is to test the impact of alternative phylogenetic hypotheses on evolutionary inferences about HBV.

**METHODS**

We used the dataset previously analyzed by Paraskevis et al. (2015), which includes 105 full-length HBV genomes from all major genotypes. We tested two phylogenetic reconstruction methods: Maximum likelihood (ML) assuming no-molecular clock (RAxML package), and Bayesian inference (BI) assuming a relaxed molecular-clock (Beast package). The best-fitting topologies for each method were compared in both models. Analysis of substitution rate and positive selection were performed in the PAML package for both best-fitting topologies.

**RESULTS**

The ML tree suggested that genotype HBV-B is sister to all other HBV genotypes, while the BI tree suggested that HBV-F/H is sister to all other genotypes. Under the ML criterion these two topologies were not statistically different ( $P = 0.142$ ; SH-test). However, under the Bayesian criterion the topology rooted on HBV-F/H had much higher support (2Ln Bayes Factor = 53.54). While both topologies did not altered the results of positive selection tests, they resulted in different estimated for the evolutionary rate of F/H. An evolutionary acceleration was suggested for the ML topology (FHrate = 0.079; Average = 0.033), but not for the BI topology (FHrate = 0.040; Average = 0.038).

**CONCLUSIONS**

A better characterization of the evolutionary dynamics of HBV using appropriated methodology and parameters is important to understand disease progression in different human populations.

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### HEPATITIS B VIRUS (HBV) REACTIVATION FOLLOWING IMMUNOSUPPRESSIVE THERAPY IN HAEMODIALYSIS PATIENTS

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#### BACKGROUND-AIM

Reactivation of HBV on immunosuppressive therapy is a recognised problem, and carries the risk of transmission in haemodialysis units.

#### METHODS

Here we report two cases of HBV reactivation in hepatitis B core antibody (anti-HBcAb) positive haemodialysis patients following immunosuppressive therapy that led to contact tracing exercises in Royal Free Hospital, London, United Kingdom.

#### RESULTS

The first incident involved an anti-HBcAb positive, HB surface antigen (HBsAg) negative and HBV DNA negative patient with chronic kidney disease secondary to vasculitis who had received rituximab without anti-HBV prophylaxis and became HBsAg and e antigen positive with detectable HBV DNA (8121 IU/ml) while on haemodialysis. A total of 165 haemodialysis patients in the unit were potentially exposed to the index patient; of these 6 (3%) patients had chronic HBV infection and 24 (16%) had past HBV infection (anti-HBcAb+ve). 44 (33%) of the remaining 135 patients had anti-HBsAb titres >100 IU/ml and were considered immune. Anti-HBsAb titres were between 10-99 IU/ml in 41 (30%) patients and <10 IU/ml in 50 (37%) patients. These 91 patients with anti-HBsAb <100 IU/ml were offered an accelerated course or booster dose of HBV vaccine and followed up for 12 weeks by weekly HBsAg testing as per Department of Health, UK guidelines. These patients were dialysed in a cohort using designated machines for 12 weeks.

The second case was an anti-HBcAb positive, HBsAg negative and DNA negative haemodialysis patient with vasculitis who received cyclophosphamide and steroids, and later reactivated HBV (DNA 23 IU/ml). 54 dialysis patients were potentially exposed to the index case; 5 (9%) were anti-HBcAb positive and 25 (46%) had anti-HBsAb >100 IU/ml. The remaining 24 exposed patients with anti-HBsAb <100 IU/ml (5 between 11-99 IU/ml, and 19 <10 IU/ml) were followed up for 12 weeks as per guidelines.

No HBV transmission was reported at the end of the 12 week surveillance period.

#### CONCLUSIONS

Our experiences highlight the importance of anti-HBV prophylaxis and monitoring of HBsAg negative and anti-HBcAb positive patients on immunosuppressive therapy in high-risk settings such as dialysis units. Guidelines are needed to prevent HBV reactivation based on risk stratifications of different immunosuppressive agents.

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### ROUTINE TESTING FOR HEPATITIS E VIRUS (HEV) AND DEVELOPMENT OF A LOCAL PROTOCOL

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#### BACKGROUND-AIM

Hepatitis E virus (HEV) is a leading cause of hepatitis worldwide. In most cases, the acute infection is mild and self-limiting, although it can rarely result in fulminant infection and death. In recent years, chronic infection has increasingly been recognised in immunosuppressed patients. In 2014, the Virology department in Royal Free Hospital, UK introduced HEV testing in all patients undergoing investigation for abnormal liver function tests (LFTs) and/or acute liver failure. The data collected over the last three years have provided us with an insight into the extent of the HEV infection in our local population, and most likely reflect the UK-wide situation. This study was undertaken to determine the prevalence of HEV infection in a North London teaching hospital population and assess the characteristics of the affected population.

#### METHODS

A pathology database search was performed to identify patients who were tested for HEV infection at the Royal Free Hospital from January 2014 – December 2016. All patient samples were tested for HEV RNA using polymerase chain reaction (PCR), with some also tested for HEV serology. The medical notes, subsequent PCR results and reference lab reports were also reviewed.

#### RESULTS

751 patients were tested for HEV infection since the introduction of routine testing. The median age of this cohort was 47 years (range 13-95 years) with an equal proportion of men and women. HEV viraemia was diagnosed in 30 patients (4%) based on PCR detection of HEV RNA. The most common indication for HEV testing was abnormal LFTs. A large proportion of these patients were immunosuppressed (42%, n=13). Of patients with HEV genotype results, most were infected with genotype 3 (83%, n=10) which has been associated with chronic HEV infection. The remaining patients were infected with genotype 1 (17%, n=2). Immunosuppressed patients were managed according to a local protocol devised specifically for this group. This protocol describes regular monitoring of HEV viraemia in blood and stool, and includes guidance on treatment with ribavirin.

#### CONCLUSIONS

HEV infection as a cause of hepatitis should be considered in both immunocompetent as well as immunosuppressed individuals. Routine testing can improve timely diagnosis and management of this infection.

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### COMMERCIAL ANTI-HCV ASSAYS COMPARISON AIMED TO IMPROVE THE DIAGNOSTIC DEFINITION OF BORDERLINE POSITIVE SAMPLES

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#### BACKGROUND-AIM

Hepatitis C virus (HCV) serological screening represents the main tool to prevent viral transmission. Several full automated test are available, with high specificity and sensitivity, however a significant number of results give borderline signal-to-cut-off (S/CO) ratio, with an uncertain interpretation.

The aim of this work was to compare the performance of two different anti-HCV tests with the immunoblot-assay in low-positive HCV-Antibodies subjects in order to improve the diagnostic algorithm.

#### METHODS

Fifty-nine consecutive samples showing a low positivity ( $1 \leq S/CO \leq 4$ ) with currently used screening test Architect® (HCV-Ablow) (Abbott) were analyzed. They were tested with standard confirmatory immunoblot assay (Deciscan® HCV Plus, BioRAD) and with i) LIAISON® XL Murex HCV-Ab (Diasorin) and ii) VIDAS® HCV-Ab (Biomérieux). In order to analyze a possible contribution of Flavivirus cross-reactivity, the prevalence of GBV-C RNA positivity was analysed by home-made RT-PCR in the sera from HCV-Ablow subjects and in 55 HCV-neg population and 66 HCVpos subjects.

#### RESULTS

A significant correlation was observed between Architect® and LIAISON® XL ( $r^2=0.36$ ,  $p<0.0001$ ) and Architect® vs. VIDAS® ( $r^2=0.16$ ,  $p=0.0004$ ). Notably, the median of S/CO values obtained by LIAISON® XL and by VIDAS® was higher in samples with a positive Deciscan® than in those with a negative Deciscan® test ( $p<0.05$ ). Among HCV-Ablow, negative samples frequency observed with LIAISON® XL (44.1%), VIDAS® (42.4%) was similar to that reported using Deciscan® (42.4%). The frequency of samples showing positive results with LIAISON® XL (55.9%) and VIDAS® (57.6%) was similar to that showing positive/indeterminate result with Deciscan® (57.6%). Whether we consider Deciscan® as the gold standard, the specificity of LIAISON® XL and VIDAS® were 56% and 64%, while the sensitivity were 80% and 100% respectively. No significant differences were observed in the frequency of GBV-C RNA+ samples in HCV-Ablow and HCV-Ablow population.

#### CONCLUSIONS

Thus, a comparable diagnostic performance of LIAISON® XL, VIDAS® and Deciscan® in identifying non-confirmed HCV-Ablow. The frequency of GBV-C RNA was similar in HCV-Ablow patients and HCV negative subjects suggesting no cross-reactivity between these viruses.

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### THE HBeAg QUANTIFICATION :AN USEFUL TOOL FOR CHRONIC HEPATITIS B TREATMENT MONITORING

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#### BACKGROUND-AIM

A more accurate serology of chronic Hepatitis B (CHB), which includes the quantification of viral structural antigens, is acquiring an important role in the CHB clinical management. In comparison to the almost consolidated use of HBsAg quantification, few data are available about the role of HBeAg (HBeAg) quantification, a new emerging tool for diagnosis and treatment monitoring.

Aim of this study was to analyse the dynamic changes in quantitative HBeAg (qHBeAg) in a group of HBeAg (+) CHB patients treated with two therapeutic schedules.

#### METHODS

CHB patients were subdivided in two groups, as follows:

Group 1: treatment for at least one year with nucleos(t)ide analogues and then add-on with 48 weeks of Pegylated interferon2a (180 mcg/weekly);

Group 2: PEG-IFN2a (180 mcg/weekly) for 48 weeks.

qHBeAg was retrospectively measured on longitudinal frozen samples (every three months) until 48 weeks after PEG-IFN2a stop by chemiluminescent immunoassay (CLIA) technology (DiaSorin, Saluggia-VC). qHBsAg and HBV-DNA data were also collected.

#### RESULTS

A total of 12 HBeAg+ patients, 8/12 (67%) viral genotype D, were evaluated.

Baseline qHBeAg values ranged widely, from 1.18 to 3443 PEI/mL, and were well correlated with HBV-DNA levels (Spearman  $r=0.747$ ,  $p=0.008$ ), slightly correlated with qHBsAg ( $r=0.55$ ,  $p=0.077$ ), while qHBsAg and HBV-DNA did not show any degree of correlation.

HBeAg seroconversion rates were perfectly comparable between the groups 1 and 2 (both 4/6). Interestingly, baseline qHBeAg, as well as qHBsAg and HBV-DNA levels, were not different between the two groups. During therapy, qHBeAg decreased in almost all patients, but at different extent: qHBeAg decrease of  $> 1 \log_{10}$  was observed in 7/8 seroconverting patients, versus 1/4 of non seroconverting (87.5 vs 25%,  $p=0.067$ ), and continued to decrease at week24, while it remained stable in non seroconverters.

#### CONCLUSIONS

qHBeAg levels fluctuate in a wide range in HBeAg(+) patients, and they seem a reliable measure of viral replication. The kinetics of decrease during treatment seem to be able to predict the HBeAg seroconversion, which is the main therapeutic end point of treatment in HBeAg (+)-patients.

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### A POINT-OF-CARE ANTI-HCV TEST POPULATION SCREENING SHOWS LOW PREVALENCE OF ACTIVE HCV INFECTION IN SOUTHEASTERN SPAIN

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#### BACKGROUND-AIM

In Spain and other EU countries there is no recommendation for HCV population screening because of lack of reliable prevalence data. We aimed to (1) assess whether rapid diagnostic tests are suitable for population screening; (2) estimate the true prevalence of undetected HCV infection in our area to identify groups at risk of chronic HCV infection.

#### METHODS

First, a risk factor questionnaire was collected from 317 consecutive patients (anti-HCV positive n=208; anti-HCV negative with other liver diseases n=109) before performing the OraQuick HCV rapid test in oral mucosal transudate (OMT) (n=317) and fingerstick blood (n=252). Second, the proposal for free HCV infection screening using OraQuick HCV rapid test in OMT was offered by regular mail to 9,000 individuals older than 18 years old, randomly selected from the hospital assigned population.

#### RESULTS

Aim 1: In OMT, the clinical sensitivity and specificity of the OraQuick HCV rapid test (using anti-HCV serostatus as the gold-standard) were 89.9% and 100%, respectively. In fingerstick blood, the sensitivity improved to 98.8%. Among anti-HCV positive patients, the sensitivity was higher in OMT (97%) in those who were viremic compared to non-viremic patients (82.2%). There were no differences in sensitivity between viremic and non-viremic individuals when testing fingerstick blood. Finally, extension of the incubation time to 40 minutes enhanced the sensitivity, especially in OMT (up to 94.7%) and in the subgroup of non-viremic, anti-HCV+ patients (up to 90.1%). Aim 2: To date, among 835 persons included in the second phase, only 12 (1.4%) were reactive according to the OraQuick HCV test in OMT. These persons were already aware of their infection.

#### CONCLUSIONS

The OraQuick HCV test in OMT has a high sensitivity and specificity that decreases substantially in anti-HCV positive HCV-RNA negative patients with treatment-induced viral clearance. This problem can be avoided by increasing the incubation times or by the use of fingerstick blood. In our department, the true prevalence of anti-HCV+ was only 1.4%, and there were no new diagnoses made. These preliminary data suggest a different epidemiological profile in our area compared to other regions (such as the USA).

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### DETECTABLE HCV VIRAL LOAD AT WEEK 8 MAY PREDICT HIGHER RISK OF RELAPSE IN INF-FREE TREATED PATIENTS.

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#### BACKGROUND-AIM

We compared results from the COBAS AmpliPrep/COBAS TaqMan HCV Quantitative Test, v2.0 ("CAP/CTM HCV v2") with those from the cobas HCV test for use on the cobas 6800/8800 systems ("cobas HCV") in a cohort of HCV Genotype (GT) 1 or GT3 infected subjects treated with various interferon-free direct acting antiviral (DAA) treatment combinations. In a second objective, the kinetic parameters were compared between subjects who achieved sustained virologic response (SVR) or relapsed using mathematical modeling.

#### METHODS

The two tests were compared using paired EDTA plasma clinical specimens. Samples run in routine testing on CAP/CTM HCV v2 were blinded and remnants were run on cobas HCV. Method comparisons were performed by Deming Regression and Bland-Altman as implemented in SAS (v 9.3 or higher). We examined viral kinetics during treatment in all subjects who had a follow-up visit after therapy termination. We compared kinetics between subjects that relapsed and those that achieved SVR and performed a multivariate analysis to identify factors that might predict viral relapse.

#### RESULTS

A total of 1,454 samples from 252 subjects were tested on both platforms, resulting in 447 paired comparisons within the linear range on both assays. Deming Regression demonstrated high correlation between the two tests, ( $R^2=98\%$ ,  $y=1.052x-0.23$ ). Bland-Altman bias plot showed most paired data were within 0.5 log, with a mean offset of 0.01 with 95% CI [-0.02, 0.04].

Results showed overall percent agreement (OPA) between cobas HCV and CAP/CTM HCV v2 using the  $6 \times 10^6$  IU/mL cutoff was 98.6% (1504/1526) with 95% Confidence Interval (CI) of [97.8%, 99.1%]. For the  $8 \times 10^5$  IU/mL cutoff, OPA was 98.4% (1501/1526), with 95% CI [97.6% - 98.9%]. Of subjects that achieved SVR by CAP/CTM HCV v2 (defined as  $VL \leq 15$  IU/mL at post treatment week 12), OPA was 99.2% (250/252) with 95% CI [97.2%, 100%].

Of 252 subjects with a follow-up visit, 7 relapsed after treatment discontinuation, while 245 achieved SVR.

For the cobas HCV, detectable viral load at week 8 was associated with a higher chance of relapse, with Odds Ratio of 1.83, 95% CI [1.04, 3.23],  $p=0.04$ .

#### CONCLUSIONS

Correlation and Overall Percent Agreement was high between the two assays examined. Detectable viral load at week 8 and beyond was associated with higher chance of relapse.

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### A NOVEL REAL-TIME RT-PCR TESTKIT FOR DETECTION AND QUANTIFICATION OF HEPATITIS D VIRUS (HDV)

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#### BACKGROUND-AIM

Hepatitis D virus (HDV) is an enveloped satellite virus with a circular negative-sense ssRNA genome causing liver disease in both acute and chronic forms. Since hepatitis B virus (HBV) envelope proteins are required for HDV packaging, HDV cannot propagate without HBV infection. Therefore, HDV infection cannot occur in the absence of HBV. Approximately 15 million people are co-infected with HDV and HBV worldwide. In >95% of the cases the infection is acute and self-limited. But the co-infection of HBV with HDV causes more severe disease than HBV mono-infection. In general, co-infection with HDV leads to a ten-fold higher mortality in patients than infections with HBV only. HDV is transmitted by contact with blood and/or body fluids of infected persons. Even though there is no HDV vaccine, protection can be achieved by HBV vaccination. Currently HDV is grouped in 8 clades with a different distribution pattern worldwide. Since HDV infections are emerging, there is a desperate need for reliable assays to detect and monitor the abundance of HDV.

#### METHODS

Altona Diagnostics has developed a real-time RT-PCR based kit for the detection and quantification of all known HDV clades. The quantification-standards were calibrated, using the 1st World Health Organization International Standard for Hepatitis D Virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays. The kit also includes an Internal Control (IC) to control the nucleic acid extraction procedure and to control for possible RT-PCR inhibitory effects.

#### RESULTS

The kit has been verified by determining the analytical sensitivity (LoD), specificity, linearity and precision and validated by analyzing proficiency panels (Hepatitis D Virus EQA Pilot Study/HDV 16; QCMD) as well as clinical isolates.

#### CONCLUSIONS

The kit described provides a reliable and validated real-time RT-PCR based kit for the detection and quantification of hepatitis D virus to be used in laboratory diagnostic and monitoring.

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### EVALUATION OF THE 3RD WHO INTERNATIONAL STANDARD FOR HEPATITIS A VIRUS (HAV) FOR NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT)-BASED ASSAYS

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#### BACKGROUND-AIM

Assuring the assay sensitivity for Hepatitis A Virus is critical in the safe manufacture of biotherapeutic medicines derived from human blood. As such the provision of calibrated reference material is critical. An International Standard for HAV established by WHO-ECBS has been available since 2003, this paper describes the study to evaluate the replacement 3rd WHO International Standard for use in the standardization of nucleic acid amplification techniques (NAT).

#### METHODS

Two candidate materials derived from a HAV subtype 1B -positive mini plasma pool, spiked into negative human plasma were assessed. One candidate was lyophilised following the addition of 40mM hepes buffer and 2% trehalose whilst the other remained plasma only. Additional study samples included pre lyophilisation liquid preparations, an HAV positive clinical plasma samples and a low titre working reagent. All materials were assessed alongside the current (2nd) WHO IS for HAV NAT.

Samples were evaluated by 11 laboratories in 10 countries, using a range of quantitative and qualitative assays. Each laboratory followed a set protocol and was requested to assay all samples on at least three separate occasions. Data were collated and analysed at NIBSC.

#### RESULTS

In total 12 data sets were received 8 from qualitative assays and 4 from quantitative assays. Data were reported by Commercial assays (8 laboratories) and Laboratory Developed Tests (LDT) (4 laboratories).

In both candidate samples the relative potency was 0.5 Log10 higher in qualitative assays vs quantitative. With potency values for the plasma only sample estimated to be 4.49 and 4.28 Log10 IU/ml for qualitative and quantitative respectively. Similarly, potency values for the candidate containing hepes and trehalose gave potency estimates of 4.62 (qual) and 4.36 (quan) Log10 IU/ml

#### CONCLUSIONS

Both candidates demonstrated the ability to harmonise a range of assays. It will be proposed to the WHO in October 2017 that the candidate containing human plasma only be established as the 3rd WHO International Standard. The majority of HAV assays report in a qualitative format, therefore it will be proposed that the materials will be assigned a potency based on the qualitative data set resulting in an assigned potency of 4.49 Log10 IU/mL when reconstituted in 0.5mL of nuclease free water.

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### EVALUATION OF TWO COMMERCIAL ASSAYS FOR DETECTING HEPATITIS E VIRUS RNA USING THE VERSANT KPCR MOLECULAR SYSTEM

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#### BACKGROUND-AIM

Hepatitis E Virus (HEV) infection is increasingly an important cause of hepatitis in developed countries. HEV sequences have been classified into four major genotypes (1-4). Genotype 3 strains are an emerging concern for immunocompromised patients, as these strains can lead to chronic infection and cirrhosis. Performance evaluations of different tests for the quantification of HEV RNA are therefore important for clinical care. In this study, we compare the performance of two commercially-available HEV quantitative PCR assays using the VERSANT® kPCR Molecular System\* with VERSANT Sample Preparation 1.0 Reagents.

#### METHODS

HEV RNA was extracted from plasma samples with the VERSANT kPCR Molecular System and VERSANT Sample Preparation 1.0 Reagents. Two commercially-available assays, the quanty HEV RT-PCR kit\*\* (Clonit, Italy) and the FTD HEV RT-PCR kit\*\* (Fast-Track Diagnostics, Luxembourg), were evaluated according to the manufacturer's instructions, analyzing the following reference materials: 1) The 1st WHO standard for HEV RNA (code 6329/10, genotype 3a) dilution series, 2) WHO reference genotype panel (PEI code 8578/13) representing all four genotypes and a range of subtypes, and 3) Qnostics Evaluation Panel of known genotypes. An additional clinical sample evaluation was performed testing 35 HEV-positive plasma samples of known genotypes.

#### RESULTS

The 1st WHO standard: The detection limits for Clonit and FTD HEV PCR kit are 100% at 125 IU/mL and 50% and 33% at 50 IU/mL, respectively. All genotypes included in the WHO reference panel were successfully detected by both assays, showing that both assays amplify genotypes 1, 2, 3, and 4. Both assays performed well analyzing the Qnostics panel. The Clonit assay detected 31 of 34 clinical samples and the FTD assays detected 33 of 35 clinical samples.

#### CONCLUSIONS

Overall, the HEV RNA assays running on the VERSANT kPCR Molecular System revealed comparable detection rates and quantification levels with reference material.

\*VERSANT is a registered trademark of Siemens Healthcare Diagnostics Inc. All other trademarks are the property of their respective owners. Product availability varies by country.

\*\*CE-marked for IVD use.

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### PERFORMANCE OF A NEW CHEMILUMINESCENT ASSAY TO DETECT AND QUANTIFY ANTI-HBS ANTIBODIES

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#### BACKGROUND-AIM

Quantification of anti-HBs antibodies (HBsAb) is a key parameter to assess vaccination efficacy or to monitor hepatitis B immunoglobulin protection level in liver transplanted patients. The aim of this study was to assess the performance of Liaison XL Murex Anti-HBs Plus (DiaSorin), a newly available HBsAb quantification technique.

#### METHODS

563 serum samples were analyzed in parallel in one laboratory using both the new technique from DiaSorin on Liaison XL and a routinely used method (Roche, Elecsys anti-HBs II) on Cobas e601 system. Titration accuracy and linearity were evaluated using WHO standard and high titrated sample dilutions (n=5). Tested samples were either from daily routine testing (n=228), selected on the basis of their complete serological profile (HBsAg, anti-HBs, anti-HBc) (n=217), or blindly provided by DiaSorin (n=118). Most discordant results were confirmed by a third technique (Vidas, BioMerieux). All results were expressed as IU/mL.

#### RESULTS

Overall the qualitative agreement between both techniques was 96.6% [94.8-97.8] and only 19/563 samples were found discordant. Additional testing of the discrepant samples with the Vidas method did not favor one technique over the other. On the WHO standard dilutions, both assays showed very good linearity with highly accurate obtained values, indicating good standardization. Linearity assessed on 10 dilutions of 5 different samples was similar between both assays. Results from 246 samples were within the quantification ranges of both techniques. The correlation was excellent (Spearman  $r=0.945$ ) with a mean bias of 8.4 IU/mL (DiaSorin-Roche) which corresponded to a mean 4% increased quantification of DiaSorin over Roche. No significant difference of quantification was observed according to the samples serological profiles.

#### CONCLUSIONS

Extensive evaluation of the new LIAISON XL HBsAb assay indicate that it provides accurate quantification and detection of HBsAb whatever the serological profile of the sample. Good standardization between assays is important particularly when samples are analyzed on different platforms.

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### EVALUATION OF A NEW REAL-TIME RT-PCR ASSAY FOR HEPATITIS D VIRUS RNA

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#### BACKGROUND-AIM

HDV RNA monitoring is an important tool for the management of HDV infection. However, commercial assays for the detection and quantification of HDV RNA are scarce. In this study, we evaluated the performance characteristics of a new HDV RNA real-time PCR assay (Bosphore HDV Quantification kit, Anatolia Geneworks, Turkey).

#### METHODS

RNA extraction was done by QIAAsymphony DSP virus kit. Assay was carried out using RotorGene real-time PCR system. The lower limit of detection (LLoD) of the new kit was evaluated using Motakk HDV RNA secondary standard derived from WHO 1st International Standard. The intra- and inter-assay coefficients of variation was evaluated by 4 plasma samples. Each sample tested three times within a run for intra-assay precision while 2 samples tested one time on three different days for inter-assay precision. Accuracy tested by 9 OCMD EQA panel samples. Specificity is determined by analyzing 20 negative samples including 7 samples positive for different viruses (HBV, HCV, CMV). Linearity analyzed by 10-fold dilution series of two positive samples tested on two different days. A comparison study was done by testing 31 HDV RNA positive patient sera singly by laboratory's existing assay (HDV Real-TM Quant, NLM, Italy) and the new evaluated kit.

#### RESULTS

The LLoD of the Bosphore HDV kit was 218 copies/ml. The intra- and inter-assay coefficients of variation ranged between 0.81 to 5.20% and 0.47 to 1.13% respectively. None of the HDV RNA negative samples were found positive. All of the six HDV RNA positive OCMD samples were detected correctly; differences between the expected and the measured viral loads (log10) were between +0.39 and -0.18. Regression coefficients (0.9982 and 0.9892), verifies the linearity of the assay throughout the range tested. The comparison study showed a reasonable correlation (R: 0.7051) in viral load values detected by two commercial assays. The difference between the assays was between 0.03 to 3.71 log10 copies/ml. The difference was  $\leq 0.5$  log10 in 38%,  $\geq 1$  log10 in 41% of the samples.

#### CONCLUSIONS

Bosphore HDV kit is a sensitive, specific, accurate and highly reproducible assay. There was a significant quantification difference between Bosphore and NLM, HDV RNA kits, which may cause problems if these assays are used interchangeably for monitoring the viral load.

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### HEPATITIS B SURFACE ANTIGEN LOSS AND 8 YEARS CLINICAL OUTCOMES WITH ENTECAVIR THERAPY IN KOCAELI

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#### BACKGROUND-AIM

We evaluated HBsAg loss in CHB patients on entecavir treatment since 2009.

#### METHODS

From January 2009 to January 2017, we treated 50 adult patients with chronic hepatitis B (compensated CHB) using entecavir. These CHB patients were HBsAg positive and HBV DNA > 2000 IU/mL for a minimum of 6 months. Eighteen (36%) patients were HBeAg positive and 32 (64%) were anti-HBe positive. These percentages were similar to those reported for the country in general. Two patients had chronic kidney disease and were on haemodialysis.

#### RESULTS

Thirty one patients underwent liver biopsy with the results of fibrosis 1 in 5, fibrosis 2 in 10, fibrosis 3 in 10, fibrosis 4 in 5 and fibrosis 5 in 1. Prior to entecavir, 26 patients had received pegylated interferon for 48 weeks, 12 had received lamivudine, 5 had received adefovir, 2 had received telbivudine and 3 had received tenofovir. In one patient HBsAg and anti-HBs were positive and an S gene mutation was demonstrated. The reason for switching to entecavir was breakthrough in 12 patients and tenofovir intolerance in 3 patients. Among breakthrough patients we detected that 5 had lamivudine resistance YMDD mutation. One patient was diagnosed with pulmonary embolism and another patient with nasopharyngeal carcinoma during treatment. Neither required cessation of entecavir treatment. The entire group remained with HBV DNA >2000 IU/mL. No patient experienced HBsAg seroclearance or progression to HCC. Two patients had HBeAg seroconversion, but one of them reconverted again in one year.

#### CONCLUSIONS

We did not found any resistance to entecavir and patients tolerated well. For HBsAg loss we follow up the patients.

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**RELATIONSHIP BETWEEN VIRAL LOAD AND HEPCIDIN IN HEPATITIS B PATIENTS**M. Altindis<sup>2</sup>, F.G. Aslan<sup>2</sup>, K. Yilmaz<sup>2</sup>, T. Demiray<sup>1</sup>, M. Koroglu<sup>2</sup><sup>1</sup>Clinical Microbiology Lab, Sakarya University Training and Research Hospital, Sakarya, TURKEY<sup>2</sup>Sakarya University Faculty of Medicine, Department of Medical Microbiology, Sakarya, TURKEY**BACKGROUND-AIM**

Increased iron storage has an important role in the diseases associated with Hepatitis B virus (HBV). Hecpidin is a hormone which functions as a key regulator in iron homeostasis and is produced in the liver. Understanding the regulation of hepcidin in chronic viral hepatitis (CVD) associated diseases may explain the relation among viral hepatitis, iron accumulation and hepatocellular carcinoma to a great extent.

**METHODS**

The study included the serum samples, which were sent to Sakarya University Medical Faculty Medical Microbiology laboratory and routinely tested and then stored at -80 ° C, from 60 patients who were followed up with chronic hepatitis B infection and serum samples from 20 healthy volunteers. The participants were divided into four groups according to their status of using medication and level of viral load ( $\geq 105$  IU/mL was accepted as high). The serum hepcidin levels in the samples of the study were investigated with the method of ELISA test (Boster, Pleasanton CA).

**RESULTS**

Of the patients included in the study, 27 were female and 33 were male with the mean age of  $42.4 \pm 6.3$  years. The hepcidin levels of the control group and patient groups are illustrated in the table. The hepcidin levels in the samples of the control group were found to be higher than those of all patient groups. However, it was detected to be higher in those with the viral load  $< 105$  IU/mL compared to those with the viral load  $\geq 105$  IU/mL.

**CONCLUSIONS**

There are few studies on hepcidin levels in patients with HBV infection, and their results are inconsistent. Although the determination of hepcidin levels by immunoassays and mass spectrometry has begun to be used more, accurate and easy hepcidin quantitation remains as a problem. Since our study had cross-sectional design, the prognostic value of low prohepcidin in CVH could not be assessed.

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**HUMAN PEGIVIRUS IN DONOR AND DIALYSIS PATIENTS; ITS RELATIONSHIP WITH HEPATITIS C VIRUS**M. Altindis<sup>2</sup>, F.G. Aslan<sup>2</sup>, S. Sipahi<sup>3</sup>, A.B. Genc<sup>3</sup>, M. Koroglu<sup>1</sup><sup>1</sup>Clinical Microbiology, School Of medicine, Sakarya University, Sakarya, Turkey<sup>2</sup>Clinical Virology and Microbiology, School Of medicine, Sakarya University, Sakarya, Turkey<sup>3</sup>Department of Internal Medicine, Nephrology, Sakarya University, Faculty of Medicine, Sakarya, TURKEY**BACKGROUND-AIM**

Of the genera of Hepacivirus and Pegivirus, Hepatitis C virus (HCV) and human pegivirus (HPgV) are the only members which are known to cause infections in humans. HPgV, which is quite common, was detected to be viremic during blood donation in 1-4% of blood donors. However, the prevalence of HPgV in HCV-infected individuals was reported to be even higher. In this study, the presence of HPgV and its coexistence with HCV in blood donors and dialysis patients were investigated.

**METHODS**

The sera of a total of 48 donors, who admitted for blood donation, underwent routine tests and whose sera were stored in appropriate conditions, including 10 anti HCV positive and 38 healthy donor serum sera, and the sera of a total of 46 dialysis patients, including 18 anti HCV positive and 28 HBV / HCV negative serum sera were included in the study. The presence of HPgV was investigated by the primer probe designed by cDNA synthesis (Viva 2 steps RT PCR Kit) after extraction from sera samples (GF-1 Viral Nucleic Acid Extraction Kit).

**RESULTS**

The presence of HPgV was not detected in the serum samples from 38 healthy donors included in the study. Whereas it was detected in one (10%) of 10 blood donors with HCV positivity. As for the group of dialysis patients, the presence of HPgV was shown in a total of four sera (8.7%) and only one of these sera was found to be HCV positive. The presence of HPgV in HCV positive patients was determined to be 6% in our study group.

**CONCLUSIONS**

Although HPgV has not been proven to be the cause of any human disease, in some studies, an association was observed between the viremia of HPgV and increase in the risk of non-Hodgkin's lymphoma. Also, the HCV RNA levels in the liver tissues of the individuals with the co infection of HCV-HPgV were found to be consistently high. For these reasons, investigating HPV in blood donor screenings can be considered, especially in the populations with its high prevalence.

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# ANTI-HCV SCREENING BEFORE SURGICAL PROCEDURES, TO PROTECT THE SURGICAL TEAM OR TO DETECT NEW TREATABLE PATIENTS

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## BACKGROUND-AIM

After the availability of the direct acting antivirals, success rate of the treatment of hepatitis C virus (HCV) infection has come close to 100%. However, several patients remain to be diagnosed and await well designed screening policies. Unfortunately, none of the countries have developed an ideal HCV screening program. In Turkey, most of the surgeons empirically perform hepatitis serology in patients before surgery. We aimed to determine the prevalence of anti-HCV and HCV RNA positivity in patients who were screened for hepatitis C before surgical procedures and to investigate the rate of the patients who are unaware of his illness.

## METHODS

Anti-HCV tests requested from all surgery clinics from 2012 to 2015 were reviewed and all positive patients were investigated whether they were HCV RNA positive, and if they have been treated. Hospital records were screened for HCV RNA positivity. Medicine reports were reached in Medula System (an integrated database from Social Security System) to collect the data of treatments. Untreated anti-HCV positive patients were called whether they were aware of being positive.

## RESULTS

During 4 years, 19627 patients were screened for anti-HCV positivity. 158 patients (0.8%) were found to be positive (76M, 82F; age: 58.2±17.6). HCV RNA was tested in 83 patients, in whom 36 were HCV RNA negative, 15 were positive and treatment-naïve, 22 positive and treatment experienced or treated after detection, 10 were followed in other centers. Seventy-five patients had no HCV RNA assay. These patients were telephoned and asked whether they were aware of anti-HCV positivity. Of 58 patients who could be reached, only 6 knew that they were anti-HCV positive.

## CONCLUSIONS

Among 19627 patients undergoing surgical procedure, 0.8% were anti-HCV positive which is the same as general Turkish population. HCV RNA negativity was higher among these patients, mostly due to previous hepatitis C treatment. Surprisingly, almost half of the patients were not tested for HCV RNA and most of the patients were not informed that they needed to be tested and followed for HCV RNA. Pre-surgical screening of anti-HCV seems not to yield catching new patients. Surgeons should be warned how important to detect new hepatitis C patients, beyond protecting themselves.

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# STATISTICAL ASSESSMENT USING SIX-SIGMA SHOWS SUPERIOR QUALITY CONTROL RESULTS FOR ABBOTT REALTIME HBV, HCV, AND HIV-1 ASSAYS

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## BACKGROUND-AIM

Six-Sigma is a universal statistical tool to assess the quality of processes. In molecular diagnostics, it can be applied to compare HBV, HCV and HIV-1 viral load assays. The Sigma metric equation combines the impacts of inaccuracy and imprecision: Sigma metric = (TEa - Bias)/CV. A 6-Sigma level indicates World Class performance with <3.4 defects per million compared to 1-Sigma level (Unacceptable performance) with 158,655 defects per million.

## METHODS

We retrospectively evaluated our quality control data obtained with RocheCAP/CTM HBV, HCV, and HIV-1 (4437) and Abbott RT HBV, HCV, and HIV-1(1455) assays. Data comprises results of assay controls (AC), commercial quality controls and laboratory-internal quality controls (LIQC). We assessed and compared the analytical performance of both applying the 6-Sigma method.

## RESULTS

For Abbott HBV, HCV and HIV-1 assays, the analysis comprised the following numbers of test results: 110-180 for high-positive (hpos) ACs, 110-180 for low-positive (lpos) ACs, and 162-257 for LIQCs at different concentrations, respectively. Across all ACs and LIQCs analyzed, a 6-Sigma level was achieved. For Roche HBV, HCV, and HIV-1 assays, the analysis comprised the following number of test results: 434-752 for hpos ACs, 398-705 for lpos ACs, 63-97 for the commercial quality controls, and 156-228 for LIQCs at different concentrations, respectively, only half of the LIQCs achieved a 6-Sigma level while the other LIQCs yielded Sigma levels between 3 and 5. Similarly, the commercial quality controls yielded 3- to 4-Sigma levels and the ACs 2- to 5-Sigma levels.

## CONCLUSIONS

The Roche assays, showed variable performances. Only a few LIQCs at higher concentrations could achieve a 6-Sigma level, while lower concentrations only yielded 2- to 5-Sigma levels. On the contrary, the Abbott RT assays demonstrated World Class performances (6-Sigma levels) independent of the control sample type or assay. Even at low viremic concentrations close to the limits of quantification. A high sigma level reflects a high degree of assay precision and accuracy that is needed for reliable results at clinical decision points.

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# DETECTION OF HEPATITIS DELTA VIRUS TOTAL ANTIBODIES IN HUMAN SERUM AND PLASMA WITH THE NEW FULLY AUTOMATED ASSAY FOR LIAISON® ANALYZER FAMILY

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## BACKGROUND-AIM

Hepatitis delta virus (HDV), identified in 1977, is the smallest human RNA virus. HDV infection can cause severe liver disease that may include fulminant liver failure and rapid progression to cirrhosis and hepatic decompensation, as well as an increased risk of liver cancer. Since HDV can only cause infection in the presence of HBV, it was thought that the widespread introduction of HBV vaccine would ultimately result in decreased prevalence of HDV. However, current studies have shown that ongoing high prevalence remains in many parts of the world, with 15–20 million people estimated to be HDV infected. The diagnosis of HDV infection relies upon the detection of total anti-HDV antibodies in patients who are HBsAg-positive. IgM antibodies are positive in acute infection and persist in chronic infection. However, they are lacking in some African patients. HDV infection should be then confirmed by the detection of serum HDV RNA.

## METHODS

The LIAISON® XL Murex Anti-HDV assay uses chemiluminescence immunoassay (CLIA) technology for the qualitative detection of total antibodies to hepatitis D virus (anti-HDV) in human serum and plasma samples. The assay results, in conjunction with other laboratory results and clinical information, is intended to be used as an aid in the diagnosis of HDV infection and as a screening test for organ and tissue donors. 1,995 specimens from an anti-HDV negative population (blood donors, HBsAg positive carriers, pregnant women, patients affected by non-HDV infectious diseases, subjects with autoimmune diseases or other diseases related to disorder of immune system, subjects from a HDV diagnostic routine) have been used to assess diagnostic specificity, while 174 specimens from an expected anti-HDV positive population have been tested for sensitivity.

## RESULTS

The diagnostic specificity was 99.35% (95% C.I.: 98.89 – 99.66%); No negative results were observed in the positive population studied with a diagnostic sensitivity of 100% (95% C.I.: 97.90 – 100%).

## CONCLUSIONS

The LIAISON® XL Murex Anti-HDV assay showed high performance in the detection of total anti-HDV antibodies. Moreover the availability of a suitable tool for screening represents the optimal starting point for the correct management of HDV infected-patients.

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# ACUTE INFECTION WITH HEPATITIS E VIRUS GENOTYPE 3 IN A 40 YEARS-OLD IMMUNOCOMPETENT WOMAN WITH GRAVE'S DISEASE: A CASE REPORT

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## BACKGROUND-AIM

Infection with hepatitis E genotype 3 (HEV3) in industrialized countries is usually a self-limiting illness. Most HEV3 infections are either asymptomatic or unrecognised. The highest icteric illness rate is recorded among men older than 60 years. A number of extra-hepatic manifestations have also been described associated with acute and chronic HEV3 infection, including autoimmune thyroiditis. The present report describes a severe acute icteric hepatitis in an immunocompetent, middle aged woman with Grave's disease, an immune system disorder that may result in an overproduction of thyroid hormones (hyperthyroidism). An association between the acute HEV3 infection and woman autoimmune background is suggested.

## METHODS

**CASE PRESENTATION:** On December 2015, a 40-year-old woman presented with jaundice to the Emergency Unit of a Hospital in Lisbon, Portugal. One week before she started with nausea, abdominal pain, having dark urine and pale coloured stools. She had a medical history of Graves' disease and an autoimmune atrophic gastritis. The admission diagnosis was acute hepatitis.

## RESULTS

Antinuclear antibodies were positive but an autoimmune hepatitis was discarded since all other autoimmune markers were negative. Blood tests showed elevation of hepatic enzymes and the higher ALT compared with AST supported the presumptive diagnosis of viral hepatitis. Serum was tested for the hepatitis virus panel and was negative for all viruses, but HEV IgM/IgG were not searched for. Instead, RNA HEV was detected in serum by RT-qPCR targeting the ORF2 region. HEV sequence was characterized using a nested RT-PCR with amplification within the ORF 1. Genetic characterization indicated that it was HEV3. Woman's clinical evolution was favourable and she was discharged 14 days after admission.

## CONCLUSIONS

Although HEV3 acute hepatitis is more frequently observed in males > 60 years old the present clinical case reports an acute jaundice/hepatitis in a middle aged woman. Although she had a Graves' disease in remission we hypothesize that her autoimmune background could have been responsible for the severe HEPATITIS that required 14 days hospitalization. To our knowledge this is the second report suggesting an association between acute HEV3 infection and hyperthyroidism.

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### CURRENT IMMUNE STATUS OF HBV VACCINEES IN HEALTH CARE WORKERS AND MEDICAL STUDENTS IN ALEXANDRIA

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#### BACKGROUND-AIM

Hepatitis B (HB) vaccine, the first vaccine to prevent cancer, has been available since early 1980s. It's safe and effective. Long term immunity is a debate. The aim of the study was to detect prevalence of protective anti-HBs level ( $\geq 10$  mIU/ml) in previously vaccinated health care workers (HCWs) and medical students. Also, determine the effect of a booster dose of recombinant HB vaccine on humoral and a marker of cell mediated immune response in subjects with non protective anti-HBs level.

#### METHODS

The study included 60 HCWs and 60 medical students from Alexandria Main University hospital. HBsAg was assayed for all subjects. Anti-HBs level was detected in HBsAg negative subjects. Serum anti-HBs and Interferon levels were assessed using ELISA after administration of a booster dose of recombinant HB vaccine to subjects with non protective anti-HBs level.

#### RESULTS

90% of HCWs and 23.3% of medical students were seroprotected. The age of 20-29 years at primary vaccination of HCWs, showed statistically significant association with protective anti-HBs level ( $P=0.044$ ). After administration of HB vaccine booster dose, 83.3% of HCWs and 97.8% of medical students showed protective anti-HBs levels. 50% of boosted HCWs and 54.3% of boosted medical students showed increased Interferon level. Statistically significant increase in anti-HBs level was detected in both groups ( $P=0.028$ ,  $P<0.001$ ), while the increase in interferon level was significant in medical students only ( $P=0.01$ ).

#### CONCLUSIONS

Post vaccination anti-HBs level should be assessed 1-2 months after the third HB vaccine dose, especially for high risk groups as HCWs. Serologic testing may be recommended for compulsory vaccinated medical students before they start their medical training. For those with anti-HBs  $<10$  mIU/mL, a single booster dose of HB vaccine should be administered and anti-HBs reassessed one month later.

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### RECENT FOODBORNE HEPATITIS A OUTBREAKS IN BUDAPEST

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#### BACKGROUND-AIM

Alimentary related infections are caused by several types of infectious agents, among which viruses are one of the most common cause. Incubation period of viral food poisoning is relatively short, infections are mainly self-limited, and the illness resolves within a short time window of generally less than 1 week. Food poisoning by hepatitis A virus (HAV) is characterized by an incubation period usually between 2-6 weeks, and a persistence of the illness for up to 8 weeks after the onset of symptoms. The virus is transmitted primarily through feco-oral route by consuming contaminated water or food or by contact with an infected person.

#### METHODS

Here we give account of a retrospective enumeration and analysis of HAV seropositive patients admitted to our hospital between January 2015 and December 2016.

#### RESULTS

During this period, we observed 2 major outbreaks due to hepatitis A infection. Both outbreaks occurred in Budapest or in the agglomeration, the first taking place in September 2015 with a rapid course, and a second outbreak with a somewhat more prolonged course in September to November 2016. During these periods we observed a prominent increase in the total number of requests for HAV serology blood tests as well as in the number of HAV seropositive patients confirmed by conventional routine ELISA method, compared to the annual average number of requests (151 and 132 vs.  $84 \pm 26$  and  $82 \pm 22$  in 2015 and 2016, respectively) as well as HAV seropositivities (50 and 56 vs.  $12 \pm 13$  and  $12 \pm 14$  in 2015 and 2016, respectively). Alimentary background was proven in both outbreaks, and the majority of the cases could be traced back to an Asian restaurant and a street gyros buffet, respectively as common point of origins.

#### CONCLUSIONS

These recent foodborne HAV caused outbreaks highlight the importance of strict surveillance and observation of food safety and hygiene regulations regarding fast food restaurants by the responsible authorities and call for higher awareness and the necessity of vaccination in the general population.

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# CHARACTERIZATION OF OBI PREVALENCE AMONG VACCINATED CHILDREN FROM ALBORZ GENERAL POPULATION, IRAN; VERTICAL OBI, MYTH OR TRUTH?

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## BACKGROUND-AIM

Occult hepatitis B infection (OBI) has been described in various clinical settings; however, studies on the prevalence of OBI among immunized general population are scarce. The real position of occult HBV in immunized populations is not well known.

## METHODS

. 558 sera samples obtained from children between 7 and 15 years old selected randomly from different schools located in Alborz Province, Iran, who already had completed doses of HBV vaccine according to standard schedule. None had received HBIG. All were checked by HBV serology and real time PCR. The parents of OBI-positive subjects were investigated by the same methodology

## RESULTS

Mean age was 8.5 years old. All subjects were negative for HBsAg and anti-HBc. In terms of anti-HBs, 300 (53.7%) and 258 (46.3%) were contained adequate (>10 IU/mL) (group I) and inadequate (<10 IU/mL) (group II) levels. 47 (15.6%) and 7 (2.7%) had OBI in groups I and II, respectively. Upon recalling of parents of OBI-infected children, 30 (64%) and 0 (0%) of either mother or father were positive for OBI related to groups I and II, respectively. None of parents were positive for HBsAg. 30.4% of OBI-positive parents had anti-HBc

## CONCLUSIONS

Anti-HBs raised by HBV vaccine alone is not enough to neutralize the HBV DNA in vertical (or perhaps intrafamilial HBV) transmission. Inadequate anti-HBs induced by vaccination alone could protect against hepatitis B disease and chronic infection, but may favor occult infections. Further molecular investigation based on high throughput next generation sequencing is undergoing.

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# PREVALENCE OF HEPATITIS E ANTIBODIES AMONG PERSONS DEPRIVED OF LIBERTY, IN THE CITY OF PAZARDJIK, BULGARIA: FIRST STUDY FOR THE COUNTRY

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## BACKGROUND-AIM

An increased serum prevalence of antibodies against HEV (anti-HEV) has been described in the last decade for Europe. At the same time studies on HEV infection in persons deprived of liberty as a risk population, are very limited. The aim of the present study is to analyze the prevalence of anti-HEV in these persons in the penitentiary institution in the city of Pazardjik in Bulgaria, as well as the correlation with other host and viral risk factors.

## METHODS

Forty from a total of 400 male persons deprived of liberty were tested for anti-HEV IgM and IgG, HAV, HCV and HBV with ELISA assay in the NRL of Viral Hepatitis. The participants underwent an interview for any risk behavior.

## RESULTS

The mean age of the studied population was  $41.1 \pm 12.29$  years. Twenty-four (60%) were Bulgarians and 16 (40%) – were of Roman ethnicity. The mean time for the stay in the penitentiary institution was  $3.18 \pm 3.86$  years. Comorbidities were detected in 22 (55%) of the inmates and past hepatitis infection were reported by 15 (37.5%) of whom 6 reported HAV, 2 – HCV, 3 – HBV, and 4 – unknown hepatitis. Alcohol consumption was reported by 20 (50%) and drug use – by 3 (7.5%) inmates. Presence of anti-HEV was established in 8 (20%) inmates of whom 4 (50%) were anti-HEV IgM and IgG positive and 4 (50%) – were anti-HEV IgG positive. The presence of anti-HEV IgG increased by age (53.25 vs. 43.06 years,  $p = 0.033$ ), and was common in those who reported alcohol consumption (35.3% vs. 8.7%,  $p = 0.038$ ). All with HEV positive serology were anti-HAV IgG positive and 1 was with a probable occult HBV (anti-HBc positive). During the 3 month follow-up period, significant changes in anti-HEV IgM titer were not established. All anti-HEV IgM positive inmates were with normal AST/ALT values, and 2 were with liver cirrhosis. None of the inmates reported contacts with livestock before arrest.

## CONCLUSIONS

Antibodies against HEV were detected in 2% of inmate population from the Pazardjik penitentiary institution. The old age and alcohol consumption were the socio-demographic factors related with anti-HEV IgG positivity. The present study is the first one of its kind in Bulgaria and further studies on the epidemiology of HEV infection among inmate population is needed.

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### HEPATITIS E VIRUS AND SERUM LEVEL AMINOTRANSFERASES IN BLOOD DONORS

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#### BACKGROUND-AIM

Hepatitis E virus (HEV) infection is a self-limiting viral infection that can lead to severe complications and death. In different regions the epidemiology of this infection varies. In this study we evaluated the seroepidemiology of hepatitis E infection in Jahrom, a city in southern Iran.

#### METHODS

This was a cross-sectional descriptive study of serum samples from 477 subjects, including 30 females and 447 males. HEV immunoglobulin G (IgG) and immunoglobulin M (IgM) were measured by enzyme-linked immunosorbent assays (ELISA). Alanine transaminase (ALT) and aspartate transaminase (AST) levels were also determined. Four hundred forty-seven subjects were male and 30 were female. Subjects were classified by age and sex.

#### RESULTS

One woman (3.3%) and 25 men (5.5%) were positive for HEV antibodies (IgG and/or IgM). There was found an association between serum level of aminotransferases and seropositivity for HEV.

#### CONCLUSIONS

The result of this study indicates that HEV is an etiological factor for hepatitis in this area of IRAN. The cost benefit of active immunization in endemic regions should be evaluated because an outbreak could have tragic consequences.

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### ANTI-HEPATITIS E ANTIBODY IN HEMODIALYSIS PATIENTS

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#### BACKGROUND-AIM

Hepatitis E Virus (HEV) is a significant health problem that causes hepatitis in developing countries. Even though, the common route of transmission of HEV is fecal-oral, other probable modes of transmission in endemic areas include vertical transmission, blood transfusions, person-to-person contact and zoonotic transmission. To date, different studies have reported controversial results about the seroprevalence of HEV in hemodialysis (HD) patients and in most of the studies increased prevalence of HEV is noted. Herein, we report our experience of anti-HEV IgG seroprevalence in a selected population of HD patients in our hospital hemodialysis unit.

#### METHODS

The study is performed between the dates August 2016-November 2016 in serum samples of 68 patients (male n=40, female n=28) on maintenance HD, sent to Ege University serology laboratory for hepatitis screening. The mean age of the HD subjects was 49.2± SD 16.8 years (age range: 19-83). Tests for the HEV serum marker (anti-HEV IgG) were carried out using a commercially available enzyme-linked immunosorbent assay kit (Euroimmun Anti-Hepatitis E Virus (HEV) ELISA IgG, Germany). Medical records were reviewed, and information on age, sex, HD duration, and evidence of hepatitis B or hepatitis C infection was collected.

#### RESULTS

The mean duration of HD treatment was 37.9 months (ranging between one month- 240 months). Among the studied individuals, the ELISA results demonstrated the existence of anti-HEV IgG antibody in only one patient and in two patients indeterminate results were obtained repeatedly in two different runs. Three (4.5%) of the patients were infected with Hepatitis B (HBV) and 11 (16.4%) with hepatitis C virus (HCV). As a result, in our study, seroprevalence of anti-HEV IgG is 1.5% (4.3% with the grayzone results) which is lower than the prevalence (6.6%) in our region.

#### CONCLUSIONS

In our study, seroprevalence of anti-HEV IgG is 1.5% (4.3% with the grayzone results) which is lower than the prevalence in our region 6.6% (13). In our patient population HCV seroprevalence is quite high (16.4%) compared to the low rate of HEV infection. The low rate of HEV may be due to the small number of patients.

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# **SERO-EPIDEMIOLOGICAL STUDY OF HEPATITIS E VIRUS AMONG THALASSEMIA AS HIGH RISK PATIENTS: A CROSS-SECTIONAL SURVEY IN JAHROM, SOUTHERN, IRAN.**

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## **BACKGROUND-AIM**

Hepatitis E virus (HEV) could be cause of viral hepatitis in the developing countries and cause severe epidemics. According to other studies, blood transfusion as a probable route of HEV infection has been suggested. An infection with hepatitis agents such as HEV causes active liver failure in multi-transfusion patients in particular thalassemia. The purpose of this study determines the seropositivity of anti-HEV antibodies in thalassemia individuals in Jahrom.

## **METHODS**

In a cross-sectional study, sera from 110 thalassemia were collected between 2013 and 2014. Enzyme-linked immunosorbent assay (ELISA) method was performed to detection of anti-HEV antibodies. Individuals' data were collected such as, demographic and clinical, for statistical analysis.

## **RESULTS**

Our results show that 10% and 1.8% of the enrolled patients were HEV Ig-G and Ig-M positive antibodies respectively. In addition, there was statistically significant difference in age groups for prevalence of anti-HEV Ig-G ( $P = 0.01$ ). Also the serum levels of liver enzymes such as ALT and AST in the HEV Ig-G and Ig-M positive samples were significantly higher than anti-HEV negative samples. But there were no significant difference between sex and splenectomy with anti-HEV positive samples.

## **CONCLUSIONS**

The results indicate more study are needed to assess HEV screening of blood products to these patients that those have a probably risk of exposure to HEV especially in higher years old.

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# **HEPATITIS E VIRUS INFECTION IN HEMODIALYSIS PATIENTS: A SEROEPIDEMIOLOGICAL SURVEY IN JAHROM, SOUTHERN IRAN**

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## **BACKGROUND-AIM**

Hepatitis E virus (HEV) is mainly the causative agent of waterborne epidemics, but some authors have found that patients on chronic hemodialysis have an increased risk of exposure to HEV. We conducted this study to reveal HEV seroprevalence in hemodialysis patients as a specific group in Iran, and to evaluate age, duration of hemodialysis, and the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in them.

## **METHODS**

The presence of immunoglobulin G antibodies to HEV(anti-HEV IgG) was measured by enzyme-linked immunosorbent assay (ELISA) in the patients' sera. Both ALT and AST serum levels were measured. The duration of hemodialysis and the age and sex of the participants were obtained from the medical records of patients, and the data were made into quantitative variables, which were expressed as mean  $\pm$  standard deviation (SD).

## **RESULTS**

43 patients (29 males and 14 females) enrolled in this study. 3 of these patients (7% of the sample) were HEV antibody positive (2 males and 1 female). The mean levels of AST and ALT in all of the studied patients were  $22.3 \pm 23.3$  IU/L and  $21.3 \pm 27.6$  IU/L, respectively. An association between HEV positivity and duration of hemodialysis was revealed by our results, but there was no significant association between HEV antibody positivity and patient age. All 3 patients who were positive for anti-HEV antibody in our study also had elevated liver enzymes.

## **CONCLUSIONS**

The finding that HEV infection was associated with elevated liver enzymes in patients who were on chronic hemodialysis may indicate that hemodialysis is a route for HEV transmission, and more controlled studies are needed to explore this association in Iran.

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# MOLECULAR PREVALENCE AND CLINICAL IMPORTANCE OF TORQUE TENO VIRUS INFECTION IN THALASSEMIA PATIENTS AS HIGH RISK INDIVIDUALS

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## BACKGROUND-AIM

Recently a novel DNA virus (Torque Teno Virus (TTV) has been identified in Japan and shown to be associated with elevated amino transferases levels after transfusion. However the exact role of TTV in pathogenesis of liver disease is yet to be established. The purpose of this study was to determine the prevalence of TTV in thalassemic patients and its relationship with elevated alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST).

## METHODS

This cross-sectional analysis study was conducted on 452 thalassemic patients. Serums were collected from all of the patients, first ALT and AST levels were determined. Then, after DNA extraction, TTV DNA was amplified and detected using semi-nested PCR, followed by gel electrophoresis. Demographic characteristics and clinical data were collected from each participant for statistical analysis.

## RESULTS

The findings showed that 160 of 452 (35.4%) samples had TTV DNA detected by PCR. From 160 TTV DNA positive, 98 (61.20%) were female and 62 (38.80%) of them were male ( $P=0.549$ ). The mean ALT and AST values in TTV positive group were higher than in TTV negative group, and the difference was statistically significant ( $p<0.0001$ ).

## CONCLUSIONS

The result showed that the prevalence of TTV in thalassemic patients in Jahrom is less than other studies in Iran and the mean ALT and AST values in TTV positive individuals were about 2 times more than in TTV negative individuals.

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# DIAGNOSTIC HBV INFECTION, BOOSTER IMMUNITY

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## BACKGROUND-AIM

Hepatitis B virus (HBV) infection remains a global public health concern. Occult HBV infection is recognized as a possible phase of chronic HBV infection.

We aimed to elucidate the significance of isolated antibodies to hepatitis B core antigen (anti-HBc) by monitoring the immune response to hepatitis B vaccination

## METHODS

The study was conducted at Infectious Disease Department, "St George" University Hospital, Plovdiv (Bulgaria) in 2016. Three patients (a mother and her two sons) diagnosed with hepatitis A and their four family closed contacts were included.

Serological markers for hepatitis A, hepatitis B and hepatitis C were tested by ELISA, using commercial kits. HBV DNA was detected by PCR (detection limit 9 IU/mL).

Closed contacts without antibodies to HBsAg (anti-HBs) received hepatitis B vaccine and their anti-HBs level were tested later. A secondary response was defined as development of an anti-HBs  $\geq 50$  mIU/mL after one dose and a primary response -  $\geq 10$  mIU/mL after 3 doses.

To further clarify the HBV status of the mother, we administered her a single dose hepatitis B vaccine. Anti-HBs were measured 1 month and 2 months later

## RESULTS

The three patients with hepatitis A were found to be exposed to HBV, with the younger brother diagnosed with inactive chronic hepatitis B 2 years ago. Further, the elder brother was also diagnosed with inactive chronic hepatitis B during a 6-month follow-up. Their mother had repeatedly reactive anti-HBc as the sole marker of HBV. Her PCR HBV DNA was negative.

The three vaccinated contacts responded with secondary immune response but the fourth contact without any HBV markers didn't. She received a second vaccination series.

The mother failed to respond to the single vaccine dose. The fact that she had two children with chronic HBV and her nonresponsiveness to vaccine were strong circumstantial evidence for chronic hepatitis B, probably occult. Liver biopsy and testing liver for HBV DNA by PCR will be diagnostic but that would not be ethically justifiable.

## CONCLUSIONS

Our results have confirmed previous data that monitoring the response to vaccination could be worth conducting not only to establish primary or secondary immune response, but also to elucidate a patient with probable chronic hepatitis B.

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# **MOLECULAR EPIDEMIOLOGY AND CLINICAL IMPORTANCE OF TT VIRUS INFECTION IN HAEMODIALYSIS PATIENTS, SOUTH OF IRAN**

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## **BACKGROUND-AIM**

Patients on hemodialysis are considered to be at risk of infection by blood-borne viruses and a prevalence of Transfusion transmitted infection has been reported in patients on hemodialysis in many countries. According to the lack of data about the prevalence of TTV in Jahrom (a city in south-west of Iran), this study was conducted to investigate the molecular prevalence of TTV viremia among hemodialysis patients in this south-west city of Iran.

## **METHODS**

In this cross sectional study serum samples from HCV and HBV negative 711 patients on maintenance hemodialysis for molecular prevalence of TT virus in south of IRAN, April, 2013. Serum samples taken before dialysis from each subjects were tested for molecular and biochemical analysis. Some possible risk factors of TT virus infection including: age, gender, duration of hemodialysis treatment and serum aminotransferases (AST and ALT) levels were collected from each studied population. Data were analyzed by use of parametric and non-parametric analyses with SPSS for Windows.

## **RESULTS**

TTV infection was detected in 27.80% of the patients. In haemodialysis patients, no association was found between TTV infection and the demographic parameters (age, sex), but we found statistically significant difference were present between these groups for what concern time on haemodialysis therapy, ALT and AST levels.

## **CONCLUSIONS**

The prevalence of TTV infection among hemodialysis patients reported by other authors is similar to our or even higher. According to the finding of present study TTV is presented as one of probable agent of hepatitis in haemodialysis patients.

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# **DRAMATIC IMPROVEMENT IN FIBROSIS SCORES FOLLOWING TREATMENT FOR HEPATITIS B AND C IN PATIENTS WITH ADVANCED LIVER DISEASE**

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## **BACKGROUND-AIM**

Antiviral therapy can suppress hepatitis B DNA indefinitely and effective oral agents are now available for hepatitis C. Patients with advanced liver disease may be treated with these agents, but the extent and time course of any improvement of their liver disease is variable and established cirrhosis was originally considered to be largely irreversible. The introduction of non-invasive fibroscan testing allows serial assessment of liver fibrosis. This study reviews a series of patients with chronic hepatitis B and C where their advanced liver disease showed dramatic improvement following the introduction of effective antiviral therapy.

## **METHODS**

A review of patients attending the hepatitis clinic with advanced liver disease (F3/F4 fibrosis/cirrhosis) resulting from chronic hepatitis B or C who had serial assessments of fibrosis by liver biopsy or fibroscan assessment. Patients whose assessment of liver fibrosis returned to normal levels post-treatment were included in the study.

## **RESULTS**

13 patients were identified in total. 9 patients had chronic hepatitis B, 3 had chronic hepatitis C and one had both chronic hepatitis B and C co-infection. Every patient had F3/4 cirrhosis prior to treatment but went on to have normal fibroscan scores following treatment.

## **CONCLUSIONS**

The study demonstrates the value of serial fibroscan assessment. Advanced liver disease and cirrhosis may be completely reversible following effective antiviral treatment in chronic hepatitis B and C. Time course of improvement can be dramatic, occurring over a period of months and provides evidence for remodelling of liver fibrosis following treatment. The residual risk of hepatocellular carcinoma (HCC) is uncertain.

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**HIV SCREENING : IT'S THE ELEPHANT IN THE ROOM**T. Vrouchos <sup>1</sup>, V. Srirathan <sup>1</sup>, F. Tariq <sup>2</sup>, J. Paul <sup>1</sup><sup>1</sup>Pennine Acute Hospitals NHS Trust<sup>2</sup>Salford Royal NHS Foundation Trust**BACKGROUND-AIM**

According to Public Health England's 'HIV testing in England: 2016 report', 13,500 (95% confidence interval 10,200-17,900) people were estimated to be unaware of their HIV infection. The UK National Guidelines for HIV testing, 2008 suggest that all adult patients with predefined clinical indicator diseases should be offered HIV testing.

**METHODS**

We performed a series of clinical audits in 4 NHS Trusts across Northwest England to establish the HIV testing rates in patients with a number of clinical indicator diseases. In particular, we looked at patients with invasive pneumococcal disease, viral meningitis, cerebral abscesses, campylobacteriosis, salmonellosis and shigellosis.

**RESULTS**

The main finding of these audits is that the HIV status of a large percentage of patients with these indicator diseases is not known. Of the patients that had the test already or were offered the test, a high diagnosed prevalence of HIV was observed in some of these patient groups, especially in shigellosis.

**CONCLUSIONS**

Our audits demonstrate that testing for HIV in certain patient groups can lead to an increased number of early HIV diagnoses, therefore leading to better patient outcomes, lower treatment costs and reduced public health implications. This audit specifically looked at the clinical indicator diseases with a bacterial or viral aetiology. It is our opinion that clinical microbiologists and virologists should incorporate this in routine clinical consultations and practice. All laboratory confirmed cases of these microbial indicator diseases should ideally include an automated laboratory comment suggesting HIV screening and should become part of management of these diseases.

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**USE OF A BACULOVIRUS EXPRESSION SYSTEM, UTILISING A CMV PROMOTER, FOR GENERATION OF VIRUS-LIKE PARTICLES IN MAMMALIAN CELLS**L. Faqih <sup>2</sup>, P. Klapper <sup>2</sup>, T. Blanchard <sup>1</sup>, P. Valley <sup>2</sup><sup>1</sup>Clinical Research Group, Liverpool School of Tropical Medicine, Liverpool<sup>2</sup>Faculty of Biology, Medicine, and Health; Division of Infection, Inflammation, and Respiratory Medicine, University of Manchester, Manchester**BACKGROUND-AIM**

Baculoviruses can be used as vehicles to efficiently deliver and express genes in mammalian cells. BacMam technology uses a recombinant baculovirus engineered to contain a mammalian expression cassette for transgene expression in mammalian cells. The mammalian gene is expressed without baculovirus replication. Virus like particles (VLPs) can be produced using this expression system. VLPs are replication-incompetent virus shells that resemble an intact, non-replicative virion lacking a genome. They maintain the original antigenic composition of the packaging component, incorporated into the virion's outer membrane. In this study, we aim to develop retrovirus-like particles to serve as a new gene therapy carrier system. Our VLP is derived from simian immunodeficiency virus (SIV) and pseudotyped with vesicular stomatitis virus (VSV) glycoprotein. The resultant hybrid VLP will be used to deliver anti-HIV IgG1 b12 antibody genes into the mammalian genome, to produce durable, high titres of neutralizing antibody.

**METHODS**

Target genes were cloned into four altered transfer plasmids, to construct recombinant baculoviruses containing the Tat/Gag/Pol genes of SIV, plus the VSV glycoprotein. All genes are driven by a CMV promoter. Confirmation of cloning performed by using unique restriction enzymes and by sequencing. Recombinant baculoviruses generated by homologous recombination between baculovirus DNA and the transfer plasmids. Mammalian cells were transduced with recombinant baculoviruses to express proteins of interest to be detected by Western blot and Elisa.

**RESULTS**

All genes of interest cloned successfully into a baculovirus transfer vector (pOET6 BacMAM). Proteins expression were detected using western blot and Elisa.

**CONCLUSIONS**

We believe that the BacMam construct will deliver SIV genes into mammalian cells and produce SIV like particles pseudotyped with VSV glycoproteins. Pseudotyping the SIV like particles with VSV-G can overcome the limited tropism of the SIV envelope genes. This strategy raises the possibility that IgG1 b12 antibody genes can be delivered and inserted into the genome of numerous cell types, to produce long-lasting, high titres of neutralising anti-HIV monoclonal antibody. Since baculoviruses cannot replicate in mammalian cells, this system can be used in vivo as well as in vitro.

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# USE OF NEXT GENERATION SEQUENCING (NGS) TO INVESTIGATE THE OCCURRENCE OF MINORITY VARIANTS INVOLVED IN ANTIRETROVIRAL RESISTANCE IN NEWLY DIAGNOSED HIV-1 PATIENTS

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## BACKGROUND-AIM

Drug resistance mutations (DRMs) remain a challenge for the treatment of HIV-1 infected patients with antiretroviral therapy (ART). Identification and surveillance of transmitted drug resistance mutations (TDRMs) circulating among therapy naïve patients is therefore an important public health concern. Current methods to detect DRMs relies on Sanger sequencing, which can detect DRMs constituting 10% to 20% of the viral population. NGS methods have made it possible to detect minority variants (MVs) occurring at a much lower frequency. Here, we analyzed if TDRMs could be detected by NGS from newly HIV-1 diagnosed therapy naïve patients.

## METHODS

Samples from 31 newly HIV-1 diagnosed therapy naïve patients were used in this study. The POL gene was amplified in duplicate and a NexteraXT library was generated for sequencing on the Illumina MiSeq benchtop sequencer. The ENVA-2016 HIV POL quality control panel from QCMD was used to identify the appropriate sequence analysis settings in CLCbio. Consensus sequences at 1% and 10% thresholds were analyzed for DRMs and surveillance drug resistance mutations (SDRMs) by using the HIVDB v.8.3 algorithm and compared to earlier Sanger sequencing results.

## RESULTS

The most concordant results with the ENVA 2016 panel sequences were obtained after trimming reads with the modified Mott-trimming algorithm at a setting of 0.01. The DRM, T215TN and the DRM/SDRM, T69TADN, causing potential to intermediate levels of resistance against some Nucleoside Reverse Transcriptase Inhibitors (NRTI) were identified in 1%-duplicates from two patients respectively, and the DRM, V106VA, causing high levels of resistance against some Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) were identified at 10%. Sanger sequencing identified none of the DRMs/SDRM. It was observed that the reproducibility of MV detection decreased with lower viral load and that sequences from late presenters (<350 CD4+ cells/mL) contained more deleterious signals.

## CONCLUSIONS

This shows that NGS can enhance the detection of TDRMs among therapy naïve patients, which could benefit patient treatment and improve the surveillance of TDRMs. However, it also indicated that reproducibility and interpretation of results could rely on viral load and the duration of HIV infection at the time of analysis.

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# NEW HIV GENOTYPING ASSAY BASED ON NEXT GENERATION SEQUENCING FOR HIV DRUG RESISTANCE TESTING

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## BACKGROUND-AIM

Since the first commercially available HIV drug-resistance tests were launched in-house assay underwent significant improvements. The emergence of next generation sequencing (NGS) platforms allowed parallel deep sequencing of clinically relevant regions with high accuracy. Here we present validation data from the first commercially available NGS-based HIV genotyping assay specifically developed for routine diagnostic use in comparison to in-house genotyping analysis (Sanger and NGS).

## METHODS

We used the Sentosa® SQ HIV Genotyping Assay (Vela Diagnostics) covering the HIV protease (PR), reverse transcriptase (RT) and integrase (IN) genes. The system comprised of 1) a robotic liquid handling system for RNA extraction and NGS library preparation; 2) Ion Torrent-based NGS system; 3) kits for RNA extraction, HIV NGS library preparation and sequencing, and 4) data analysis and reporting software. The Vela system allows sequence data export for usage of alternative data interpretation systems. For comparison all samples were analysed with our in-house HIV genotyping system using Sanger and NGS for genotyping. Subtype prediction of HIV Vela system was compared to our in house system using the COMET HIV-1 subtyping tool. Resistance interpretation of the HIV VELA system as well as the exported sequencing data were compared to an in-house Sanger and NGS-based analysis using the HIV-GRADE interpretation system.

## RESULTS

We analysed 122 plasma samples with a viral load of more than 1000 copies/mL of therapy naïve and therapy experienced patients with 3 different HIV genotyping methods. Not all samples could be amplified, however 109 samples could be successfully analysed with all three methods. With regard to subtype prediction between Vela versus Sanger we saw a concordance of 97.3% within PR-RT and 96.3% within IN. The detected resistance mutations were highly concordant between Vela and Sanger and between Vela and in-house NGS.

## CONCLUSIONS

Vela Diagnostics provides a highly automated HIV genotyping platform based on next-generation sequencing with impressive fast sample analysis in only 3 days. The concordance of the results between Vela, in-house Sanger and NGS was very high.

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# DETECTION OF DRUG RESISTANCE MARKERS IN HIV-1 USING THE QIAACT HIV-1 NGS GENOTYPING KIT IN COMBINATION WITH THE GENEREADER(TM) NGS SYSTEM

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## BACKGROUND-AIM

Human immunodeficiency virus type 1 (HIV-1) is a lentivirus, that if not treated, over time leads to acquired immunodeficiency syndrome (AIDS). Antiretroviral therapy (ART) can be used to slow the progression to AIDS but HIV-1 can rapidly develop resistance which leads to treatment failure and continued viral replication.

The capability to detect drug resistance mutations in HIV-1 infected patients has been part of routine standard of care for many years. It is a vital tool in maximising treatment options, controlling the prevalence of drug resistant viruses and improving patient management.

The novel QIAact HIV-1 NGS Genotyping Kit is currently being developed to detect drug resistance markers in HIV-1 infected individuals directly from plasma using the GeneReader NGS System. This study demonstrates HIV-1 subtype coverage and performance of the assay in comparison to Sanger sequencing. It also gives a full definition of the complete QIAact HIV-1 NGS Genotyping system.

## METHODS

Contrived samples with known mutations were tested using the QIAact HIV-1 NGS Genotyping system and the correlation between the defined mutational status and the detected mutations was determined. Additionally, the 2nd WHO International Reference Panel Preparation for HIV-1 Subtypes for NAT (NIBSC code: 12/224) was tested to determine subtype coverage. The sequence data was analysed using a customised version of the CLC Biomedical Genomic Workbench software. The automated bioinformatics solution includes a single workflow, which based on a resequencing approach, detects mutations that according to the Stanford database are known to be associated with drug resistance.

## RESULTS

The QIAact HIV-1 NGS Genotyping Kit detected the expected subtypes from the WHO International Reference Panel Preparation for HIV-1 Subtypes. The assay detected mutations known to confer drug resistance in contrived samples and was furthermore shown to be more analytically sensitive than Sanger sequencing with respect to the mutant frequency detection level.

## CONCLUSIONS

The QIAact HIV-1 NGS Genotyping Kit demonstrated broad inclusivity of HIV-1 subtypes and detection of drug resistance mutations in well-characterized samples.

Disclaimer: The QIAact HIV-1 NGS Genotyping Kit using the GeneReader® NGS System is currently under development.

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# THE PRACTICAL FEASIBILITY AND CLINICAL UTILITY OF A ROUTINE NEXT GENERATION SEQUENCING SERVICE FOR THE DETECTION OF HIV ANTIVIRAL DRUG RESISTANCE MUTATIONS IN A MODERN NHS HOSPITAL DIAGNOSTIC LABORATORY

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## BACKGROUND-AIM

Drug resistance testing is an integral part of the management of HIV infected patients who are receiving antiretroviral therapy (ART) as it informs the treating clinician on the optimal treatment regimen for that patient. The aim of this study was to assess the practical feasibility and diagnostic utility of using a next generation sequencing (NGS) platform for HIV genotyping. For this the Vela diagnostics automated Ion Torrent based platform with the Sentosa SQ HIV assay was used.

## METHODS

Sixty samples from HIV infected patients being treated for HIV at University Hospital Leicester (UHL) were tested. The sixty patients included 14 patients who were known to be on treatment, 28 treatment naïve patients, 8 patients who had previously been on treatment and 10 of the patients treatment history was unknown. Viral loads were tested for each of the patients on the Abbott m2000. These samples were sent to the Birmingham reference laboratory for Sanger sequencing and NGS on the Vela diagnostics platform was completed at UHL. Sequencing was performed on the reverse transcriptase, protease and integrase gene regions.

## RESULTS

The viral loads of the patients ranged from 2.43 to 7 log<sub>10</sub> copies/ml. Sanger sequencing and NGS sequencing was successful for all samples tested. The subtypes of the HIV viruses, based on the Sanger results, from these patients included A1 (n=2), B (n=22), C (n=25), CRF02\_AG (n=3), CRF-06\_CPX (n = 1), D (n = 2), G (n =3) B,G recombinant (n =1) and one sample was unassigned based on Sanger sequencing. All subtypes were concordant between Sanger and NGS sequencing. Discrepant sequencing results, for variants detected above 20% of the viral population, were observed in 10% of the samples tested. Viral drug resistance or accessory mutations below 20% (minority variants) of the viral population were detected in 33% (n=19) of the samples tested. Where information on treatment history was available it was assessed whether the NGS sequencing results would have had an impact on patient management compared to the Sanger sequencing results.

## CONCLUSIONS

Vela diagnostics NGS sequencing was found to be non-inferior to the Sanger method but with the added benefit of detecting minority drug resistance variants.

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# RESISTANCE ASSOCIATED MUTATIONS TO INTEGRASE STRAND TRANSFER INHIBITORS IN HIV-1 NON-B GENETIC VARIANTS VIRAL ISOLATES IN THE CENTRAL REGION OF PORTUGAL

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## BACKGROUND-AIM

Resistance associated mutations (RAMs) to integrase strand transfer inhibitors (INSTIs) may occur as natural polymorphisms. Likewise, the tendency for mutation selection may differ between HIV-1 genetic variants. However, scarce clinical data are available concerning the impact of HIV-1 genetic variant on the susceptibility to INSTIs. The objective of this study was to assess the prevalence of INSTIs RAMs in HIV-1 non-B viral isolates from INSTIs treatment-naïve individuals in the central region of Portugal.

## METHODS

Nucleotide sequences of the integrase coding region of the pol gene (codons 1-288) of 32 HIV-1 non-B viral isolates from infected individuals with no prior exposure to INSTIs, between 2015 and 2017, were retrospectively analysed. HIV-1 genetic variants were identified using the REGA HIV-1 Subtyping Tool. The prevalence of INSTIs RAMs was estimated according to the 2017 update of the International Antiviral Society mutations list and the assessment of the impact on INSTIs susceptibility was done using the Stanford University HIV-1 genotypic resistance interpretation algorithm.

## RESULTS

Of the 32 nucleotide sequences analysed, 17 (53.1%) were identified as subtype G, 6 (18.8%) as sub-subtype A1, 5 (15.6%) as subtype C, and 4 (12.5%) as unique recombinant forms. The T97A polymorphic accessory mutation was the only INSTIs RAM observed, with an overall prevalence of 3.1%, and it was associated with a subtype G viral isolate. Of note, it was found a prevalence of 18.8% amino acid substitutions not associated with resistance to INSTIs but occurring at some accessory resistance codons (L74I/V, E138D, and R263S).

## CONCLUSIONS

The results of this study suggest that the prevalence of naturally occurring polymorphisms associated with resistance to INSTIs is low in HIV-1 non-B viruses circulating in the central region of Portugal. Moreover, no major INSTIs RAM was observed, and alone, the only RAM observed has minimal impact on INSTIs susceptibility. Nevertheless, it was found a significant prevalence of amino acid substitutions with a still unclear potential effect on INSTIs susceptibility due to its occurrence at some accessory resistance codons, and therefore, further in vitro and clinical studies are needed.

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# IN SEACH FOR A RAPID ALGORITHM IN THE DIAGNOSIS OF HIV INFECTION

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## BACKGROUND-AIM

This study is planned to question the availability of a fourth generation rapid test in the algorithm in Turkey as a second test where we exactly do not know the incidence of acute retroviral syndrome and seroprevalence of HIV infection for the time being to be able to perform a rapid algorithm.

## METHODS

In the first part of the study 104, 4th generation EIA reactive samples were tested by Alere Determine HIV-1/2 Combo (Alere Medical Co). Of the 104 samples 84 were WB positive, 9 negative and 11 were indeterminate /negative.

In the second part a total of 253 sera were tested by the new version Alere HIV Combo test. Of the 253 sera, 130 were screened 4th generation EIA negative sera tested for specificity whereas 123 were 4th generation EIA repeatedly reactive and WB positive for sensitivity detection.

## RESULTS

In the first part in 84 WB positive samples, 82 were Alere Determine HIV-1/2 Combo reactive (sensitivity 97.6%). Nine WB negative sera were non-reactive but 11 indeterminate or negative sera were found reactive.

In the second part with the new Alere HIV Combo all 130 4th generation EIA negative were non-reactive (specificity 100%) and 123 4th generation EIA repeatedly reactive and WB positive sera were all reactive by Alere HIV Combo (sensitivity 100%).

## CONCLUSIONS

Western blot being less sensitive than EIA is no more trusted as a confirmatory test in especially newly infected patients. In combination of WHO and CDC algorithm for a rapid diagnosis, the need for a second test with sensitivity and specificity greater than 99% was obvious especially in countries where prevalence is low or not known to increase the predictive value of the first repeatedly reactive sera in a very short time. So in addition to CDC algorithm we may also use this 4th generation rapid test as the second test and then application of quantitative RNA will both help as the third test and help in decision for starting the therapy. Time consuming western blot will be omitted in this way. This rapid cost effective algorithm should be tried on more samples and evaluated till the incidence of acute retroviral syndrome is determined and it is believed to be rapid and cost-effective omitting the western-blot step which may miss early infection and providing a sensitive and specific second step test in the rapid algorithm.

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### EVALUATION OF THE VITROS HIV COMBO ASSAY FOR THE SIMULTANEOUS DETECTION OF ANTI-HIV 1/2 ANTIBODIES AND HIV P24 ANTIGEN

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#### BACKGROUND-AIM

Recently, a new 4th generation chemiluminescence screening test (VITROS HIV Combo, Ortho-Clinical Diagnostics) has become available for the simultaneous detection of anti-HIV 1/2 antibodies and HIV p24 antigen. The aim of our study was to evaluate this assay versus the current assays, in use at our Lab: LIAISON XL HIV Ag / Ab, DiaSorin (4th gen test) and VITROS aHIV 1/2 Ortho-Clinical Diagnostics (3rd gen test).

#### METHODS

Three different groups were simultaneously tested with the three methods. Group 1: 1003 sequential samples of the daily laboratory routine. All the positive or doubtful samples underwent Western Blot (WB) to confirm the results and the HIV-RNA was sought using real time molecular biology techniques. Group 2: 50 samples from known HIV positive patients. Out of them, 25 were HIV-RNA positive and 25 HIV-RNA negative. Group 3: 50 samples present in our serum bank with indeterminate result at WB. Out of them, 24 were HIV-RNA positive and 26 HIV-RNA negative.

#### RESULTS

For the Group 1, all the three tests indicated that 988 (98.5%) were negative, and 10 (1.0%) reactive (all of them confirmed by WB; 6 were HIV-RNA positive and 4 HIV-RNA negative); 5 samples (5%) gave discordant results (4 were WB negative and 1 WB indeterminate; all of them HIV-RNA negative). The sensitivity of the VITROS Combo test is 100% and the specificity is 99.9% or 100% depending on whether the indeterminate WB is interpreted as negative or positive. For the Group 2 all the samples were reactive with all the three tests. For Group 3, 18 (36%) were negative with all the three tests, 30 (60%) reactive (24 were HIV-RNA positive and 6 HIV-RNA negative) and 2 (4%) discordant (HIV-RNA negative). The overall correlation between the three tests was 99.4%: 99.7% between VITROS Combo and VITROS 3rd gen; and 99.6% between VITROS Combo and LIAISON XL.

#### CONCLUSIONS

The VITROS Combo has proven to be highly sensitive and specific in detecting HIV infection.

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### PREPARATION AND EVALUATION OF THE 4TH INTERNATIONAL STANDARD FOR HIV-1 RNA

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#### BACKGROUND-AIM

The use of highly sensitive and accurate molecular methods for the detection of HIV is required to diagnose and monitor infected individuals and to ensure the continued safety of the blood supply. The WHO 1st International standard (IS) for HIV-1 RNA was established in 1998, its provision has allowed the continued improved agreement between NAT assays by laboratories developing a hierarchy of traceable reference materials to calibrate, evaluate and monitor assays. Here the evaluation of the 4th IS for HIV-1 RNA is outlined.

#### METHODS

A heat inactivated candidate standard, derived from stock material previously used for the 2nd and 3rd standards was assessed in an international collaborative study. 21 laboratories from 10 countries were invited to take part. The candidate material was assessed alongside the current standard. A further 4 samples were included to assess commutability, effect of lyophilisation and unit assignment of two quality control regents. All samples and laboratories were coded; each laboratory followed the same protocol and was requested to assay the samples on 3 separate occasions. All data was returned to NIBSC for analysis.

#### RESULTS

23 data sets were returned to the NIBSC with 15 different assay methods represented; of these 11 were quantitative and 4 were qualitative.

Mean estimates derived from individual laboratories values showed good correlation between the candidate material and the current standard. However such results demonstrated large inter laboratory variability (2.60–5.44 log<sub>10</sub> IU, copies or NAT-detectable units/ml).

The proposed candidate material when expressed as a relative potency to the 3rd IS demonstrated excellent harmonisation, demonstrating inter laboratory variability of 4.91–5.77 Log<sub>10</sub> IU/ml, standard deviation were also very low - 0.06% and 0.43% for quantitative and qualitative results respectively. Accelerated stability assessment shows the material to be stable when shipped ambient.

#### CONCLUSIONS

Performance of the candidate material demonstrates it to be suitable as a replacement standard. The study will be presented to the WHO Expert Committee on Biological Standardisation in October 2017 with a recommendation to be established as the 4th IS for HIV-1 NAT with a proposed unit of 5.10 Log<sub>10</sub> IU/ml.

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**COMPARISON OF ELISA, CMIA AND ELFA METHODS FOR DIAGNOSIS OF HIV INFECTION**E. Karakece<sup>1</sup>, F.G. Aslan<sup>3</sup>, M. Koroglu<sup>2</sup>, H.A. Terzi<sup>1</sup>, O. Aydemir<sup>1</sup>, M. Altindis<sup>3</sup><sup>1</sup>Clinical Microbiology Lab, Sakarya University Training and Research Hospital, Sakarya, TURKEY<sup>2</sup>Clinical Microbiology, School Of medicine, Sakarya University, Sakarya, Turkey<sup>3</sup>Clinical Virology and Microbiology, School Of medicine, Sakarya University, Sakarya, Turkey**BACKGROUND-AIM**

Many tests have been used in the diagnosis of HIV over years and with developing testing methods. The review of used algorithms periodically introduce updated recommendations and algorithms for HIV tests which also necessary for HIV diagnosis. Also, this introduces new approaches for the correct evaluation of the test results.

The aims of this study was to update the recommended algorithm for HIV testing, as a use the ELFA test, and recommend it for HIV testing and suggest approaches to evaluate the test results correctly.

**METHODS**

Patients tested for any medical reason for anti-HIV during between 1 January 2016 and 31 May 2017 was evaluated retrospectively. We evaluated 30 HIV-infected, 22 HIV-uninfected specimens. All samples were studied with the methods of Anti HIV1/2 test Micro-ELISA (Triturus, Grifols, Spain), ELFA (Vidas® HIV Duo Ultra, bioMerieux, France), Chemiluminescent Microparticle Immunoassay (CMIA) (Architect I2000, Abbott Laboratories, USA). At the same time, HIV RNA PCR (Gene Xpert, Cepheid) were also studied with the same samples. At the end of the study, reactive and Grey zone blood samples were studied with these two methods again and remaining patient samples were protected at -80°C. On the other hand, the samples having positive Anti HIV 1/2 which were determined during the iterative tests were sent to the Ministry of Health, Turkish Public Health Institution Reference Laboratory for the verification with the Western-Blot (WB) method.

**RESULTS**

All HIV RNA and WB positive samples (n = 30) were positive with Micro-ELISA, CMIA and ELFA. 22 negative samples of HIV RNA and WB were positive with Micro-ELISA and CMIA, while just 4 samples by ELFA were positive.

**CONCLUSIONS**

False positives are still a major problem despite the constant regeneration and improvement of screening tests. As can be seen from the results obtained, the false positive rate by ELFA method was found to be about five times lower than that of Micro-ELISA and CMIA methods. Considering that antigen (p24) and antibody positivity can be given separately with this aspect, it can be considered that there is a confirmation place in HIV diagnosis algorithm. However, there is a need for more extensive work to express this more precisely.

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**A QIAGEN-DEVELOPED BIOINFORMATICS PIPELINE FOR PREDICTION OF HIV-1 RESISTANCE VARIANTS USING NEXT GENERATION SEQUENCING (NGS) DATA**K. Einer-Jensen<sup>3</sup>, N. Lubke<sup>2</sup>, A. Thielen<sup>1</sup>, S. Javed<sup>5</sup>, G. Wall<sup>4</sup><sup>1</sup>Institute of Immunology and Genetics, Kaiserslautern, DE<sup>2</sup>Institute of Virology, Heinrich-Heine-University Düsseldorf, DE<sup>3</sup>QIAGEN Aarhus, Aarhus, DK<sup>4</sup>QIAGEN GmbH, Hilden, DE<sup>5</sup>QIAGEN Ltd, Manchester, UK**BACKGROUND-AIM**

The ability to predict human immunodeficiency virus type 1 (HIV-1) resistance variants has relied for many years on Sanger sequencing. However, such technology can only detect HIV variants present at approximately 10-20% of the viral population.

NGS is an alternative method to Sanger sequencing and allows mutation at lower frequencies to be detected. This study focuses on the development of a bioinformatics pipeline that automatically predicts drug resistance based on HIV-1 NGS data.

**METHODS**

A panel of 21 HIV-1 patient samples were selected, representing different HIV-1 subtypes. Some contained resistance mutations in the protease (PR), reverse transcriptase (RT) and integrase (IN) regions. RT-PCR amplicons were prepared for Illumina MiSeq using the Nextera XT library kit and in parallel for generation of Sanger sequencing data.

Three bioinformatics pipelines were applied in parallel:

1. "deeptypeHIV" in-house solution from the Institute of Immunology and Genetics
2. a customised and automated pipeline based on CLC Genomic Workbench, QIAGEN;
3. an automated proof of principal pipeline developed according to IVD software standards, QIAGEN.

**RESULTS**

Sanger consensus sequences were extracted using the CLC GWB and analysed using the online "HIValg Program" (<https://hivdb.stanford.edu/hivalg/by-sequences/>). The hereby identified "major resistance mutations" were considered as the correct result. Results of the NGS pipelines 1, 2 and 3 all agreed with the Sanger-based predicted drug resistance.

**CONCLUSIONS**

- Comparison of the three independently developed bioinformatics solutions generated equal results across the 21 diverse samples.

- The QIAGEN proof of principal solution is fully automated and delivers unified reporting aimed at users with little or no bioinformatics expertise.

- Once fully verified, the QIAGEN proof of principal solution has the potential to provide valuable biological insights as part of QIAGEN's future QIAact HIV-1 NGS Genotyping Kit using the GeneReader® NGS System.

**Disclaimer:**

The QIAact HIV-1 NGS Genotyping Kit using the GeneReader® NGS System and bioinformatics pipeline is currently under development.

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# COMPARISON OF TWO HIV-1 VIRAL LOAD QUANTIFICATION ASSAYS IN DETECTING VIRAL LOAD BLIPS IN PATIENTS WITH LONG-TERM SUPPRESSED HIV-1 VIRAL LOAD AND PATIENTS WITH PERSISTING LOW-LEVEL VIREMIA

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## BACKGROUND-AIM

High accuracy of plasma viral load [VL] determination in the low range is crucial since repeated VL blips >50 copies [cps]/ml may drive a physicians' decision to switch from well-tolerated medication to regimens with higher pill burden or toxicity. This study compares the performance of the new Roche cobas 6800 HIV-1 assay to Abbott RealTime HIV-1 in clinical specimens from long-term successfully treated patients with known history of HIV-1 VL as determined by RealTime.

## METHODS

Specimens from patients with the following VL characteristics were selected for the study: undetectable at date of analysis and >2 years in the past (Group A; N=30); <50 cps/ml at date and with a higher frequency of detected/quantified blips in the past (Group B; N=50); consistent low level viremia of 50 to 200 cps/ml (Group C; N=20). Median treatment time for Groups A, B and C was 14, 5 and 2 years, respectively. Plasma separation was performed within 4 hours from blood collection followed by storage at -80°C for up to 180 days prior to testing on both platforms.

## RESULTS

In Group A (reference result "undetectable"), no sample was found with VL >50 cps/ml with either assay; 3/30 (10%) were detectable, but not quantifiable with cobas, while one sample (3%) was detectable with RealTime, respectively. In Group B (reference results <50 cps/ml) 13/50 (26%) samples quantified >50 cps/ml with cobas, while 5/50 (10%) quantified above this threshold with RealTime. In Group C (reference results 50-200 cps/ml), 5/20 (25%) samples quantified >200 cps/ml with cobas and 2/20 (10%) with RealTime, respectively. Considering matched result pairs from samples with "detectable/quantifiable" VL, significantly higher median viral loads (Wilcoxon Signed Rank test p=0.026) were observed with cobas versus RealTime (median difference +0.12 log).

## CONCLUSIONS

No blips were observed with either cobas 6800 or RealTime in samples from patients with long-term undetectable VL. However, viral load blips occurred 2.5 times more frequently with cobas 6800 when testing plasma samples from patients with detectable but non-quantifiable viral loads <50 copies/ml (pre-tested with RealTime) for a long period of time.

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# MULTICENTRIC PRECISION ANALYSIS OF TWO FULLY-AUTOMATED HIV VIRAL LOAD ASSAYS, APTIMA HIV-1 QUANT DX AND ROCHE HIV-1 FOR USE ON THE COBAS 6800, IN COMPARISON TO ABBOTT REALTIME HIV-1

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## BACKGROUND-AIM

The subject of this evaluation was to assess the precision of the more newer generation of fully-automated HIV-1 assay generation (Hologic Aptima and Roche Cobas 6800) in comparison to the Abbott RealTime reference particularly near the clinically relevant threshold of 50 copies/ml.

## METHODS

For concordance analysis between the assays, frozen plasma samples derived from seven patients (subtypes B, C, AE, AG and A) under routine clinical monitoring (RealTime) at five time points were selected retrospectively for retesting with all three assays. For precision analysis, frozen plasma samples were diluted to a nominal concentration of 50 copies/ml (based on RealTime). Four different clinical specimens, representing the four most prevalent HIV-1 subtypes worldwide (B, C, AE and AG) were included. All samples were tested in triplicates over five days in three different labs per assay for precision analysis.

## RESULTS

Across all subtypes there were only minor differences between Aptima and RealTime in clinical follow-up samples (highest mean difference 0.29 log for subtype A). Aptima and RealTime results differed more noticeable from Cobas 6800 results particularly for subtypes C and AE in clinical follow-up samples (range 0.33 – 0.56 log). In precision analysis at 50 copies/ml mean, median and standard deviation values turned out to be higher for Cobas 6800 as compared to Aptima and RealTime for all subtypes. Mean, median and standard deviations for RealTime and Aptima differed slightly for subtype AE but were highly concordant for subtype B, C and AG replicates without passing a delta 0.06 log value. Median log difference from Aptima minus RealTime were +0.08, -0.02, +0.19 and -0.01 for subtypes B, C, AE and AG, respectively and +0.28, +0.36, +0.31 and 0.24 log for Cobas minus RealTime, respectively.

## CONCLUSIONS

The three assays show an overall good agreement around the point of decision of 50 copies/ml. All replicates were detected across all subtypes. Aptima and RealTime showed a high agreement between their results and differed more noticeable from Cobas 6800 results. As an important criterion, this may ease the interpretation of Aptima results in comparison to previous RealTime results. In this context, low viremic Cobas 6800 results after RealTime monitoring may require a closer view.

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**SEROEPIDEMIOLOGY OF HIV IN SRI LANKAN TB PATIENT COHORT**D.P.C.K.A. Lal<sup>1</sup><sup>1</sup>National Hospital of Sri Lanka**BACKGROUND-AIM**

TB and HIV co-infection is considered to occur worldwide. Immunosuppression by HIV makes patients vulnerable to be infected with TB and they are more prone to get severe disease. Prevalence of TB and HIV in Sri Lanka is 4.2% and < 0.1% respectively. Relationship between HIV and TB is not yet clearly defined in Sri Lanka. The objective of this preliminary study is to describe the epidemiology of HIV – TB co-infection in Sri Lanka.

**METHODS**

54 sexually active patients with histopathologically or microbiologically proven Tuberculosis were screened for HIV with ELISA antibody test. Positive ELISA was confirmed by western blot test.

**RESULTS**

Patients were 17 to 54 years of age. Male: Female = 33:21. 38 and 16 patients had pulmonary and extrapulmonary TB respectively. Only 02 male patients had positive ELISA test for HIV but both were negative for western blot test.

**CONCLUSIONS**

HIV – TB co-infection is not a significant occurrence in Sri Lanka yet. There for HIV should not be considered as an important predisposing factor for TB in Sri Lanka and It is not rational to screen all TB patients for HIV as it is not cost effective for a resource poor country.

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**PERFORMANCE EVALUATION OF THE COBAS® HIV-1/HIV-2 QUALITATIVE NUCLEIC ACID TEST FOR USE ON THE COBAS® 6800/8800 SYSTEMS IN EUROPE AND SOUTH AFRICA**A. Nilsson<sup>3</sup>, B. Kramer<sup>3</sup>, B. Seiverth<sup>3</sup>, P. Gohl<sup>1</sup>, S. Carmona<sup>2</sup>, R. Luo<sup>4</sup><sup>1</sup>Bioscientia Institute for Medical Diagnostics, Ingelheim, Germany<sup>2</sup>National Health Laboratory Service, Wits University, Johannesburg, South Africa<sup>3</sup>Roche Diagnostics International, Rotkreuz, Switzerland<sup>4</sup>Roche Molecular Systems, Pleasanton, USA**BACKGROUND-AIM**

Although HIV testing is universally recommended, over half of the approximately 37 million individuals with HIV do not know their status. This study evaluated the performance of the cobas® HIV-1/HIV-2 Qualitative nucleic acid test for use on the cobas® 6800/8800 Systems (cobas® HIV-1/2 qual), a new automated real-time PCR assay that detects and differentiates between HIV-1 and HIV-2 in EDTA-plasma, serum, and dried blood spots (DBS).

**METHODS**

Limit of detection was determined with WHO International Standards for HIV-1 and HIV-2. Assay inclusivity was determined on an additional 15 HIV-1 and HIV-2 groups and subtypes. cobas® HIV-1/2 qual was compared to serology assays using 153 plasma and 150 serum samples and to a state-of-the-art nucleic acid test on 148 clinical plasma and serum samples in Europe and 279 infant DBS in South Africa. Specificity was determined by testing over 1800 negative plasma, serum, and DBS samples and samples containing potential interferents.

**RESULTS**

The limit of detection for cobas® HIV-1/2 qual was verified as 12.6 cp/mL, 12.1 cp/mL, and 255 cp/mL for HIV-1 plasma, serum, and DBS, and 27.9 cp/mL, 23.4 cp/mL and 984 cp/mL for HIV-2 plasma, serum, and DBS, respectively. The assay detected all HIV-1/HIV-2 subtypes and groups tested. In seroconversion panels, the assay detected HIV an average of 8.5 days earlier than the state-of-the-art serology test. In clinical plasma, serum, and DBS samples, cobas® HIV-1/2 qual agreed with state-of-the-art serology and nucleic acid testing >99.6% of the time. Further testing on discordant samples agreed with the cobas® HIV-1/2 qual 100% of the time. Specificity was 100% with no effect seen from other pathogens or potential interferents.

**CONCLUSIONS**

The cobas® HIV-1/2 qual assay is highly sensitive and specific for diagnosing HIV-1 and HIV-2. The assay can detect HIV infection over a week earlier than serology, enabling earlier HIV treatment and prevention of ongoing HIV transmission. Identifying HIV-2 infection correctly is also critical to ensure proper treatment and viral load monitoring. HIV diagnostic algorithms should consider including HIV-1/HIV-2 nucleic acid tests.

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**PERFORMANCE OF THE LUMIPULSE® G HTLV-I/II ASSAY**T.D. Ly<sup>1</sup>, C. Coignard<sup>1</sup><sup>1</sup>Laboratory Eurofins Biomnis, Ivry sur Seine, France**BACKGROUND-AIM**

There are two types of HTLV: HTLV-I and HTLV-II. It is estimated that 15-20 million people are currently infected with human T-cell lymphotropic virus type 1 (HTLV-1) worldwide. HTLV-II is found among Native Americans. Transmission of both HTLV I and II occurs through sexual contact, exposure to blood, transfusion of infected cellular blood components and perinatally, probably by breast feeding.

In this study, the performance of a new HTLV assay (Lumipulse® G HTLV-I/II, Fujirebio) was compared to that of LIAISON® XL murex recHTLV-I/II (DiaSorin) and ARCHITECT rHTLV- I/II assay (Abbott), used routinely in our laboratory.

**METHODS**

343 unselected serum samples submitted to the laboratory for HTLV testing were examined also by LIAISON® XL and Lumipulse® assays. Samples that were discordant were tested by INNO-LIA HTLV I/II Score (Fujirebio) for confirmation. Sensitivity was evaluated using 56 frozen HTLV-I positive, 5 HTLV-II positive serum specimens (confirmation by Immunoblot INNO-LIA HTLV I/II Score) and a HTLV I/II Mixed Titer AccuSet Performance Panel (0820-0192) of SeraCare.

**RESULTS**

Among 343 routine samples, 338, 339 and 340 samples were negative with ARCHITECT, LIAISON® and Lumipulse® respectively. 3, 4 and 5 samples were reactive with Lumipulse®, LIAISON® and ARCHITECT respectively. The 3 discrepancies samples (1 and 2 weakly reactive with LIAISON® and ARCHITECT) were not confirmed by immunoblot.

Lumipulse® had an overall agreement of 99.4% (341/343) and 99.7% (342/343) with ARCHITECT and LIAISON® respectively. ARCHITECT and LIAISON® had 100% negative agreement with Lumipulse®.

Results from the Performance panel dilution series showed the greater sensitivity of Lumipulse® for detecting HTLV-I antibodies. In contrast the LIAISON® had a greater sensitivity for detecting HTLV-II. Architect was less sensitive. In addition, all 61 positive HTLV samples were detected by these 3 assays.

**CONCLUSIONS**

The HTLV assay performance of ARCHITECT, LIAISON® and Lumipulse® were equivalent. Lumipulse® G HTLV-I/II assay, performed on Lumipulse® G 1200 instrument, demonstrated very good specificity and sensitivity. It is appropriate for the screening of HTLV-1/2 antibodies.

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**INTERNATIONAL MULTI-CENTER STUDY OF 4 ROCHE HIV-1 RNA ASSAYS DEMONSTRATES COMMUTABILITY AND CONCORDANCE ACROSS DIFFERENT PLATFORMS**E. Vancutsem<sup>1</sup>, D. Pierard<sup>1</sup>, V. Michel<sup>2</sup>, H. Reiske<sup>2</sup>, M. Njoya<sup>4</sup>, J. Canchola<sup>4</sup>, E. Paxinos<sup>4</sup>, E. Marins<sup>4</sup>, J. Lau<sup>4</sup>, K. Luk<sup>4</sup>, C. Simon<sup>4</sup>, P. Cunningham<sup>5</sup>, S. Carmona<sup>3</sup><sup>1</sup>AIDS Reference laboratory, Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium<sup>2</sup>Covance Central Laboratory Services SA, Geneva, Switzerland<sup>3</sup>HIV & Haematology Molecular Laboratory WITS University - NHLS Charlotte Maxeke Johannesburg Academic Hospital, South Africa<sup>4</sup>Roche Molecular Systems, Pleasanton, CA<sup>5</sup>St Vincent's Centre for Applied Medical Research, St Vincent's Hospital Sydney, Australia and Kirby Institute for Infection and Immunity in Society, University of New South Wales, Sydney, Australia**BACKGROUND-AIM**

HIV RNA viral load (VL) is a marker of virological progression and a direct measure of the effect of antiretroviral therapy. With UNAIDS setting of the ambitious 90/90/90 targets, access to diagnosis and care is becoming a reality to millions of HIV-infected people globally, and new automated systems are being implemented in many labs worldwide to accommodate needs for higher throughput. As patients may be tested at different locations with different assays, high commutability and concordance of results better supports patient management. This multicenter study compares the commutability and concordance of results from 4 different Roche HIV-1 RNA assays: cobas HIV-1 test for use on the cobas 6800/8800 Systems (cobas 8800 HIV), cobas HIV test for use on the cobas 4800 System (cobas 4800 HIV), COBAS AmpliPrep/COBAS TaqMan HIV v2 test (CAP/CTM HIV v2), and COBAS TaqMan HIV test for use with the High Pure System version 2 (CTM/HPS HIV v2).

**METHODS**

HIV-1 limit of detection (LOD) and linearity at lower concentration were assessed across all 4 assays using panels traceable to the 3rd WHO international standard. HIV-1 RNA concordance analyses were performed by calculating mean paired log differences. Assay correlation of clinical specimens was performed using Deming regression analysis.

**RESULTS**

The 4 assays demonstrated similar LODs, and broadly overlapping 95% confidence intervals (CI); cobas 8800 HIV-1: 11.7 cp/mL (95% CI [8.9, 24.8]), HIV-1 CAP/CTM v2.0: 15.8 cp/mL (95% CI [12.0, 28.4]), CTM/HPS v2: 15.0 cp/mL (95% CI [11.2, 28.5]), and cobas 4800 HIV 29.8 cp/mL (95% CI [19.8, 67.5]). Accuracy ranged from -0.01 log cp/mL with cobas 8800 HIV to 0.12 log cp/mL with CAP/CTM HIV v2. Deming regression analyses demonstrated strong correlation between all assay-pairs ( $R^2 \geq 0.97$ ) and the mean paired log difference ranged from -0.16 log cp/mL to 0.11 log cp/mL.

**CONCLUSIONS**

This large, international, multi-centre study demonstrated commutability of all 4 quantitative HIV-1 RNA assays, across different laboratories and platforms. All tests can be used reliably in clinical practice to guide treatment decisions. Moreover, the cobas 6800/8800 and cobas 4800 systems offer higher throughput and automation than previous platforms.

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**EVALUATION OF THE VITROS IMMUNODIAGNOSTIC HIV COMBO TEST**B. Holmgren<sup>1</sup>, H. Larsson<sup>1</sup>, B. Böttiger<sup>1</sup><sup>1</sup>Division of Laboratory Medicine, Department of Clinical Microbiology, Lund, Sweden**BACKGROUND-AIM**

This study was performed to evaluate the performance of the VITROS Immunodiagnostic Products HIV Combo (Ortho Clinical Diagnostics) which received approval from the Paul Ehrlich Institute during the fall of 2016.

**METHODS**

Samples positive for HIV-1, HIV-2 or HIV-1 p24, previously identified in our laboratory by either Abbott ARCHITECT HIV Ag/Ab Combo assay or the LIASON XL Murex HIV Ab/Ag, and further confirmed with Inno-Lia HIV-I/II Score Assay, Innatest HIV Antigen and/or HIV-1 PCR, were included. In addition, international reference panels, anonymised blood donors, and serial clinical sample submitted to the laboratory for HIV-screening test, were tested.

To evaluate repeatability and precision, three different quality controls, anti-HIV-1 (Accurun 2, SeraCare Life Sciences, Milford), anti-HIV-2 (DK HIV2 ARCH -Ax 040525), and HIV-1 antigen (Accurun106, SeraCare Life Sciences, Milford) were tested.

**RESULTS**

A total of 24 HIV-1 positive, 3 HIV-2 positive, and 1 HIV-1 antigen positive samples were tested. All of the samples were reactive in the Vitros Combo test.

An international WHO standard (1st International Reference Panel NIBSC code: 02/210), containing 1:40 dilutions of plasma samples positive for anti-HIV-1 subtypes A, B, C and CRF01\_AE, anti-HIV-1 Group O, and anti-HIV-2 respectively, were all tested reactive. In addition, a CE marked material HIV-2 (antibody) monitor sample (NIBSC code: 99/674) was also tested reactive.

A serially diluted WHO international HIV-1 p24 standard (NIBSC code: 90-636) showed p24 antigen sensitivity of 0,16 IU/mL and 0,31 IU/mL for Vitros and Innatest respectively.

A total of 1172 blood donors, non-reactive in the LIASON XL Murex HIV Ab/Ag test, were tested and gave an estimate of specificity of 99,8%. None of the serial clinical samples (N=146) were reactive.

A CV% between 5.8 and 9.1 was obtained when positive controls were tested on different machines, with different reagent batches, and on different days.

**CONCLUSIONS**

Data from this evaluation suggest clinical performance of the HIV Vitros Combo test comparable with previous tests used in the laboratory.

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**PERFORMANCE EVALUATION OF A 4TH GENERATION HIV AG/AB ASSAY WITH A NEW BUFFER FORMULATION**B. Bubenik<sup>1</sup>, P. Groborz<sup>1</sup>, P. Sztolarikova<sup>1</sup>, J. Simkova<sup>1</sup>, K. Pobudova<sup>1</sup>, M. Hraske<sup>1</sup>, R. Fojtikova<sup>1</sup>, C. Drexler<sup>2</sup>, P. Schlenke<sup>2</sup>, D. Zanin<sup>3</sup>, A. Pighini<sup>3</sup>, L. Pallavicini<sup>3</sup><sup>1</sup>Blood Center Frydek-Mistek, Frydek-Mistek, Czech Republic<sup>2</sup>Department for Blood Group Serology and Transfusion Medicine, Medical University Graz, Graz, Austria<sup>3</sup>Product Development Department, DiaSorin S.p.A., Italy**BACKGROUND-AIM**

Fourth-generation assays detect both HIV antibody and p24 antigen, and are designed to reduce the window period, providing the possibility to achieve an earlier diagnosis. Combination assays have sometimes been reported to be less sensitive for detecting p24 antigen than standalone p24 assays. However, the progressive improvement of fourth-generation assays, lowering the detection limit for p24 antigen, has removed this sensitivity difference. As two different assay formats are combined in fourth-generation assays, there is a potential risk of increased nonspecific reactivity, reducing the specificity. Also in this regards, many efforts have been made by IVD companies to increase the assay specificity. The aim of this work is to evaluate the sensitivity and specificity performance of a 4th generation HIV Ag/Ab commercial assay with a new buffer formulation.

**METHODS**

The LIAISON® XL murex HIV Ab/Ag HT (DiaSorin S.p.A., Italy) is a sandwich chemiluminescence (CLIA) immunoassay for the qualitative determination of specific antibodies to HIV-1/2 and HIV p24 antigen. A change in its buffer formulation has been done, namely the addition of a detergent and buffer replacement, to achieve optimal specificity performance on fresh samples from routine donors.

Totally 7,414 fresh blood donor samples from two different European blood centers have been tested to evaluate specificity. 13 commercially available seroconversion panels, the WHO First International Reference Reagent (NIBSC code 90/636) and the HIV-1 Panel of the French Agency for Health Safety (Agence Française de Sécurité Sanitaire des Produits de Santé, Afssaps) have been evaluated for the sensitivity assessment.

**RESULTS**

The assay showed a final specificity of 99.81% (95% C.I. 99.68% to 99.90%). On the seroconversion panels, the test diagnostic sensitivity in the detection of HIV early infection was substantially equivalent to or better than the reference assay. When testing of the Ag panels, the assay showed an average value of 0.41 IU/mL with the WHO standard and 11.22 pg/mL with the French standard.

**CONCLUSIONS**

All the data confirms the suitable performance of the LIAISON® XL murex HIV Ab/Ag HT for the screening and diagnosis of HIV infection.

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### INFLAMMATION MARKERS AND LOW LEVELS VIREMIA: GENDER DIFFERENCE IN ARV TREATED HIV-1 PATIENTS

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#### BACKGROUND-AIM

HIV-positive patients (pts) with virological success show persistent immune activation/inflammation. It was shown that such activation could be associated to host factors rather than viro-immunological markers. We investigate gender difference in ART-treated HIV-1 pts about immune activation status and its relationship with residual viremia.

#### METHODS

252 HIV-1 pts [130 female (F) and 122 male (M)] were retrospectively examined for 45 months. HIV-1 RNA was measured by kPCR (Siemens). D-dimer, sCD14, C-reactive protein (CRP) and IL-6 were measured by ELISA test. Chi square and Mann-Whitney tests were used to determine difference between groups. The risk of virological failure (VF) was calculated by Kaplan-Meier method.

#### RESULTS

To evaluate the risk of virological failure, pts were divided according to basal viremia: pts with undetectable viremia (TND: Target not detected), 67 F and 51 M; pts with low levels viremia (LLV), 63 F and 71 M. The rates of VF (HIV-1 RNA >400 copies/mL) was higher in F-LLV than in F-TND (26% vs 7.5%; p=0.004) and the risk of VF in F-LLV was significantly higher than in F-TND (log rank p=0.006). No significant difference about rate and risk of VF between M-LLV and M-TND was found. The median levels of D-dimer were higher in F than in M ([208,8 ng/mL (IQR 128,2-348,4) vs 154,4 ng/mL (IQR 79,2-285,9) p=0,019]; on the contrary, median levels of CRP were higher in M than in F [CRP: 3,9 ug/mL (IQR 1,4-9,02) vs 2,47 ug/mL (IQR 0,7-5,8) p=0,013]. No difference was observed between F and M groups when sCD14 and IL-6 levels were compared [sCD14: F 2,7 ug/mL (IQR 2,0-3,8) vs M 2,5 ug/mL (IQR 1,8-3,7 p=0,095; IL-6: F 0,6 pg/mL (IQR 0,6-3,2) vs M 0,6 pg/mL (IQR 0,6-5,2 p=0,32]. Finally, the influence of viremia on inflammatory markers levels was evaluated. Interestingly, D-dimer levels were significantly different only when F with LLV were compared to M with LLV. On the contrary CRP as well as sCD14 levels were higher in M-TND group than in F-TND.

#### CONCLUSIONS

The data obtained underline the complex interaction between host and virus. Although gender seems to affect the risk of VF and activation/inflammation status, the presence/absence of a minimal viral replication may affect the difference detected. The study was supported by Gilead grants (fellowship program 2015)

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### ULTRA-POTENT NEUTRALIZATION OF PARVOVIRUS B19 BY A VP1-SPECIFIC HUMAN MONOCLONAL ANTIBODY

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#### BACKGROUND-AIM

Discovered in 1975, Parvovirus B19 is the only member of the Parvoviridae family infecting humans. In addition to the common childhood disease erythema infectiosum, B19 virus is also associated to more severe diseases, including hydrops fetalis and fetal death, aplastic crises, polyarthralgia and arthritis and pure red-cell aplasia. The current treatment for patients suffering from persistent B19 virus infection is administration of human immunoglobulin preparations (IVIG) that own limited efficacy and toxicity risks.

Aim of this study was to isolate a human monoclonal antibody (mAb) able to potently neutralize Parvovirus B19 to the goal of developing an effective therapy for pregnant woman and chronically infected patients.

#### METHODS

IgG+ memory B cells isolated from three selected cryopreserved tonsils were immortalized with EBV and interrogated for the presence of antibodies binding to VLPs displaying VP1/VP2 or VP2 alone. Positive cultures were expanded and the VH and VL sequences were retrieved by RT-PCR to produce recombinant human IgG1. Anti-VP1 and anti-VP2 IgGs were identified and tested both in competition studies and neutralization assays using seronegative patient sera from primary B19 infections containing B19 on UT7/Epo-S1 cells. After 3 days, the level of neutralization was measured by immunofluorescence analysis after permeabilization using a 488DL-anti-B19. Epitope specificity on a selection of VP1-specific antibodies was determined using a library of cyclic peptides.

#### RESULTS

Nine anti-VP2 and six anti-VP1 mAbs were isolated and used in cross-competition studies. We found that VP2-specific antibodies targeted at least 3 distinct antigenic sites, while VP1-specific antibodies reacted to at least 4 distinct sites. The neutralizing activity of this mAb panel showed a wide range of IC50. Remarkably, one mAb directed to VP1 (dubbed PAB5) neutralized B19 virus at extremely low IC50 (<2 pg/mL). Further characterization and additional neutralization studies are currently ongoing to define the epitope specificity, the breadth of reactivity and the mechanism of action of this ultra-potent neutralizing mAb.

#### CONCLUSIONS

PAB5 human IgG1 mAb potently neutralized the B19 and could be developed as a novel therapeutic agent to treat severe B19 infections.

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### SUCCESSFUL USE OF ALLOGENIC CMV-SPECIFIC T LYMPHOCYTES TO TREAT A CMV-INFECTED LYMPHOPENIC LIVER TRANSPLANTED PATIENT AND STUDY OF CMV MIRNOME TO EVALUATE VIRUS CLEARANCE

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#### BACKGROUND-AIM

Cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in organ transplantation. Management of CMV infection includes the use of antiviral drugs for prophylaxis and/or therapy and treatment options, dealing with effects as neutropenia and the emergence of GCV-resistant virus. Here we report a case of a 16 years old man, CMV-seronegative, who underwent liver transplantation from a CMV-seropositive donor. The patient developed a primary CMV infection characterized by a persisting leucopenia and interstitial pneumonia despite the treatment with antiviral drug. It is notable that no ganciclovir-resistant CMV DNA was found. Considering the serious infection relapses after antiviral treatment suspension and the developed lymphopenia, an adoptive immunotherapy approach, using heterologous T-cell was considered. Lately, many studies have focused their attention on the prognostic value of the miRNAs coded from CMV, 26 mature miRNAs, related in the regulation of different mechanisms like viral replications, immune modulation and immune evasion.

#### METHODS

Allogenic T lymphocytes from healthy CMV-seropositive donor (patient's mother) were expanded in vitro using CMV peptide-pulsed dendritic cells and then infused in a liver-transplanted patient with recurrent CMV infection in spite of GCV treatment. We profiled the viral miRNome (26 microRNAs) using microfluidic cards (qRT-PCR).

#### RESULTS

After adoptive transfer of allogenic CMV-specific T-lymphocytes the general health condition of the patient improved, viral load decreased in peripheral blood and in bone marrow and no further relapses were recorded. With regard to the miRNA expression profile in the peripheral blood, we detected 7 microRNAs that have a good correlation with the viral load. Interestingly, these microRNAs show a no relapse after the infusion of allogenic T cells, while the DNA viral load increased maybe as effect of cell lysis. This may suggest the role of the CMV miRNAs in discerning an active transcriptional event.

#### CONCLUSIONS

The generation and infusion of allogenic T cells to treat CMV infection recurrence, when a HLA-matched donor is available, is an alternative strategy when the generation of autologous virus specific T cell clones is not possible.

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### EXPANSION OF MULTIVIRUS SPECIFIC T CELLS FROM FROZEN DONOR LYMPHOCYTE INFUSION (DLI) PREPARED FOR THE PATIENT AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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#### BACKGROUND-AIM

Patients who are undergoing intensive chemotherapy followed by allogeneic stem cell transplantation are immunosuppressed and therefore are in a great risk of reactivation of viruses such as CMV, EBV, or contracting viral infection from the environment such as the human adenovirus AdV. These infections are one of the major contributors to post-transplant morbidity. Treatment options are limited to application of antivirals specific for CMV but not for EBV and AdV. The reconstitution of the anti-viral immunity is slow following allo-SCT and is even actively suppressed by application of immunosuppressive drugs to prevent the graft-versus-host disease. Therefore, the treatment of such severe viral infection represent a significant challenge to current medicine. It has been shown in a number of experimental clinical studies that it is feasible to select and expand antiviral T cells from donor lymphocytes and transfer them into patients to treat the acute viral infection. The adoptive transfer of expanded T cell lines specific for CMV, EBV, AdV and other viruses is able to produce a sustained long-term clinical response.

We have optimized conditions for expansion of virus specific memory T cells.

#### METHODS

Expanded cells were characterized by flow cytometry and by ELISPOT IFN-gamma with following results.

#### RESULTS

Two sources of donor T cells (freshly isolated PBMC from peripheral blood nad frozen buffy coats for donor lymphocyte infusions (DLI) were compared. Large amount of virus specific cells was obtained if DLI was used. The influence of various growth factors (IL4, IL7, IL21) on activity of expanded virus specific T cells has been determined. We observed that supplementation with high concentration of IL4 (1666 U/ml) increased expansion rate and IFN-gamma response of T cells in comparison with 1000 lower concentration. The influence of cultivation vessel (bottle vs. cultivator with gas permeable bottom) has been also determined. It was observed that T cell lines expanded under optimal conditions have prevalently the phenotype of effector memory CD4+ and CD8+ cells with low markers of apoptosis and contained smaller proportion of naive T cells (CD45RA+, CCR7+, CD27+).

#### CONCLUSIONS

The work was supported by grant NV15-34498A from AZV, Czech Republic.

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### CHARACTERIZATION OF EPSTEIN-BARR VIRUS BY ANALYSIS OF SHORT TANDEM REPEAT POLYMORPHISMS

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#### BACKGROUND-AIM

Epstein-Barr virus (EBV) is a  $\alpha$ -herpes virus that latently infects more than 90% of the adult human population worldwide. After primary infection which occurs mostly during childhood, EBV persists in some B cells for the lifetime. Primary infection is usually asymptomatic but is sometimes responsible for Infectious Mononucleosis. EBV is also oncogenic and its persistence can be associated with several types of cancer, including endemic Burkitt's lymphoma, post-transplantation lymphoproliferation, Hodgkin's disease, nasopharyngeal carcinoma and gastric carcinomas. EBV infection is notably a main issue in transplant recipients, since immune suppression dramatically increases the risk of EBV-associated lymphoproliferations. The aim of this work was to investigate inter- and intra-individual EBV strain variations in transplant patients with a high EBV viral load, by developing a new molecular typing method for EBV. Short tandem repeat (STR) polymorphism has been successfully used to investigate genetic variability among CMV, HSV-1 and HSV-2 strains and a similar strategy was designed to study EBV.

#### METHODS

After STR identification within the whole genome of EBV reference strain B95-8 using the MSatFinder program, sequence alignment of 7 complete EBV genomes was performed in silico to identify highly polymorphic loci. 8 chosen STR were PCR-amplified and subsequently sequenced on both strands for 38 clinical isolates from 29 patients and 5 EBV-positive cell lines.

#### RESULTS

We showed that only 5 polymorphic STR loci are sufficient to delineate the molecular genotype of EBV strains. The STR haplotype for the cell lines and the clinical isolates obtained from distinct individuals were all different. No variation in haplotypes was observed for isolates from different tissues of the same patient. In addition, STR haplotypes were stable over time, except for two patients for whom a change in EBV haplotype was observed after organ transplantation.

#### CONCLUSIONS

STR polymorphism analysis is an accurate tool to discriminate both clinical and laboratory EBV strains, providing a rapid, reliable and low-cost genotyping method for EBV. Applied to cohorts of patient with severe EBV-related neoplasia, this technique may be very useful to identify EBV isolates exhibiting a higher oncogenic potential.

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### PARTICULATE MATTER 10 (PM10) EXPOSURE INDUCES JC POLYOMAVIRUS REPLICATION

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#### BACKGROUND-AIM

Human Polyomavirus (HPyVs) are small non enveloped DNA viruses that asymptotically infect an high percentage of human population during childhood and then establish latency in the host. Reactivation can occur when the immune system is impaired.

Air pollution exposure is a major problem worldwide and has been linked to many diseases. Particulate Matter (PM)10 is one of the components of air pollution. Studies assessing the relationship between PM10/PM2.5 exposure and the replication of the HPyVs are completely lacking.

#### METHODS

A Chemical Transport Model was used to estimate daily PM10 concentrations at municipality resolution of 50 healthy adult subjects. For each subject, a fast urine sample and an EDTA tube of blood to separate plasma fraction were collected.

DNA was isolated from the urine samples and multiplex Real Time PCR were conducted to quantify HPyVs (JCPyV, BKPyV, MCPyV, HPyV6, HPyV7 and HPyV9) loads in the urine. Extracellular vesicles (EVs) were purified from plasma and concentrated by ultracentrifugation: miRNAs were isolated, reverse transcribed and the levels of JCPyV 5p miRNA were quantified by means of specific Real Time PCR assay.

#### RESULTS

HPyVs DNA was detected in 58% ( $4.9 \times 10^5$  copies/ml) of urine samples. JCPyV genome was the most prevalent (48%) with a mean viral load of  $6.6 \times 10^5$  copies/ml (range:  $1.82 \times 10^3$  -  $6.72 \times 10^6$  copies/ml).

JCPyV 5p miRNA was expressed in 7 EVs samples (14%), 4 of which collected from patients who did not show viruria.

PM10 exposure of 3, 4 and 5 days before the sample collection was associated with an increase in JCPyV genome presence in urine samples (Delta percentage were respectively 15.0%, 16.6% and 14.3%, p-values<0.01).

#### CONCLUSIONS

PM10 exposure can affect the replication of JCPyV which is urinary excreted after 3, 4, 5 days from exposure. The circulating JCPyV 5p miRNA may have a role in the downregulation of the viral replication.

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### TOWARDS ENSURING THE QUALITY OF HHV-6 DIAGNOSIS IN IMMUNOCOMPROMISED PATIENTS

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#### BACKGROUND-AIM

Opportunistic reactivation of latent Human herpes 6 virus (HHV-6) in immunocompromised patients undergoing solid organ and in particular allogeneic stem cell transplantation, can pose a significant burden leading to increased incidence of morbidity and mortality in affected individuals. Consistent patient management relies on the accurate quantification of HHV-6 viral loads and better comparability across laboratories.

#### METHODS

To address this challenge we have worked with the WHO's Expert Committee for Biological Standardisation to develop candidate HHV-6 International Standard (IS) materials for use as a primary order calibrant. We have undertaken a multi-centre international collaborative study to evaluate these preparations alongside HHV-6 positive clinical samples, with the aim of demonstrating that the candidate material is suitable for use as a primary calibrant by virtue of its ability to reduce the variability of the data across the participating laboratories and by assessing commutability by including clinical samples.

#### RESULTS

Viral load evaluations of a coded test panel were performed by 26 laboratories across 12 different countries including clinical, research and reference laboratories, IVD manufacturers, and an EQA provider. Preliminary analysis of the data illustrates an almost 3.0 log<sub>10</sub> variation in copy number reporting for the candidate materials across laboratories. However expressing the data as a relative potency in comparison to a common reference, we show a reduction in this variability down to <0.5 log<sub>10</sub> copies/ml. Using the same approach of relative potency to the evaluation of the clinical samples, the results from different laboratories were also harmonised.

#### CONCLUSIONS

The widespread application of higher order calibrants improves the comparability of NAT data between laboratories. The employment of a common unitage (International Unit IU) will enable better definition of clinically-relevant viral loads and thereby improve patient diagnosis and treatment.

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### TTV R-GENE® KIT, A NEW REAL TIME PCR ASSAY FOR THE DETECTION AND QUANTIFICATION OF TORQUE TENO VIRUS (TTV): COMPARISON WITH REFERENCE METHOD AND EQUIVALENCE BETWEEN EXTRACTION AND PCR PLATFORMS

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#### BACKGROUND-AIM

TTV is an ubiquitous DNA virus from the human virome, not yet associated with a specific disease. Its potential as an immunosuppression biomarker recently underlined by several publications, introduces the need for monitoring its viral load. We thus developed the TTV R-gene® (kit for Research Use Only, not for use in diagnostic procedures, 69-030B ARGENE®, bioMérieux) for standardized detection and/or quantification of TTV in whole blood (WB) and plasma. We present here the internally comparison of our assay to a reference assay previously used for assessment of the TTV viral load clinical value (TTV in-house PCR, Maggi et al, JCV, 2003) and equivalence results on several PCR platforms.

#### METHODS

Cloned TTV genomes of various genotypes spiked in water and nucleic acids from 23 WB and plasma samples from kidney donors or healthy volunteers extracted by NucliSENS® easyMAG® (bioMérieux) were amplified by TTV R-gene® and reference assay. Comparison of extraction and amplification instruments, easyMAG® or MagNA Pure Compact and CFX96TM, Rotor Gene® Q, LightCycler 480 and StepOne involved 3 dilutions (7, 5 and 3 log cp/mL) of genotypes 8, 12, 27 spiked in WB.

#### RESULTS

All tested genotypes (1, 3, 6, 7, 8, 10, 12, 15, 16, 19, 27 and 28) were detected by both assays. On clinical samples, the concordance was 85% with 5 discrepant samples, weakly positive with R-gene assay and negative with reference PCR. TTV viral loads obtained with TTV R-gene® were correlated in paired plasma and WB samples (mean difference=0.27 log cp/mL, SD=0.43). Compared to the combination of reference (easyMAG®/ABI7500®), all combination of extraction and amplification platforms showed equivalent results (difference <0.5log cp/mL).

#### CONCLUSIONS

TTV R-gene® assay compared with reference PCR showed equivalent performances. Correlation between plasma and WB viral load enables further use of WB as a routine sample. The protocol and format common to the ARGENE® Transplant range allows to use the kit in the same workflow and to potentially monitor the patient's immune status. The validation of several extraction and amplification platforms brings flexibility to adapt this assay in most of the labs, as part of the demonstration of the clinical utility of TTV viral load monitoring.

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### CYTOMEGALOVIRUS-SPECIFIC CELLULAR IMMUNE RESPONSE AND VIRAL REACTIVATION AFTER LIVER TRANSPLANTATION FOR CHRONIC HEPATITIS C

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#### BACKGROUND-AIM

There are several factors associated with the severity of recurrent Hepatitis C virus (HCV) infection after liver transplantation (LT), including cytomegalovirus (CMV) reactivation. Alterations in the CMV-specific cell-mediated immune response (CMI) could result indirectly in an inadequate control of HCV replication post-transplantation, because the modification in the pool of T lymphocytes. We hypothesized that an altered CMV-specific CMI could be immunopathogenic and result in an inadequate control of viral replication. Our aims were to evaluate (i) if the CMV-specific CMI can predict which patients will control CMV replication spontaneously, and (ii) if CMI is a cost-effective approach to the management of HCV and CMV infection in the LT setting.

#### METHODS

We evaluated T-cell specific responses prospectively in 25 liver transplant patients, including 16 HCV-positive at LT, and nine of them with treatment-induced resolved HCV infection before LT. PBMCs were collected just before LT and at several time points after transplantation and cryopreserved. CMV-specific CMI (T-cells) was assessed by intracellular cytokine staining (ICS) after stimulation with CMV peptide pools: pp65, IE-1 and IE-2 and a positive control (SEB) for 6hr. An 8-color flow panel was developed for ICS with CD107a-APC, TNFα-PE and IFNγ-FITC, a live/dead stain and the cell surface antibody mix (CD3-BV510, CD4-PE-Cy7, CD8-PerCPy5.5, CD69-BV421). All possible combinations of functional T-cell subpopulations were analyzed using boolean gates with the FlowJo (V10.2) software.

#### RESULTS

CMV reactivation after LT occurred in 12/25 patients (10 D+/R+ and 2 D+/R-). From them, 9/16 patients with active HCV infection at LT reactivated CMV and 8 also showed severe HCV recurrence. In baseline/pretransplantation samples, polyfunctional pp65 and IE-1, but not IE-2, specific CD8+ T-cell responses were higher in those patients who did not reactivate CMV after transplantation, and potentially associated with slow fibrosis progression after LT.

#### CONCLUSIONS

CMI ICS assays are very useful for the evaluation of CMV-specific T-cell responses and their role in the control of CMV reactivation and in the outcome of the graft after liver transplantation. Studies increasing the sample size are warranted.

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### THE DISTRIBUTION OF CMV GENOTYPES IN DIFFERENT PATIENT GROUPS

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#### BACKGROUND-AIM

Although, human cytomegalovirus (CMV) is a common pathogen which usually remains asymptomatic in the healthy persons, it can cause symptomatic disease in the immunocompromised patients. Glycoprotein B (gB), is one of the main targets of the immune response and CMV can be classified into using its sequence. The aim of our study was to investigate the CMV glycoprotein B (gB) genotypes in different clinical samples of different patient groups.

#### METHODS

Forty six patients (21 female, 25 male, aged between 0-71, mean age 34,29±20,22) found positive for CMV DNA were included to the study. CMV genotypes were determined in total 49 samples of 46 patients.

Following the purification of DNA gB gene was partially amplified by nested PCR method. The nested-PCR products sequenced bidirectionally and phylogenetic analysis was performed using partial gB sequences.

#### RESULTS

gB1 (51.03%) was found in 25 samples; gB2 (28.57%) in 14 samples, gB3 (10.2%) in 5 samples; and gB4 (10.2%) in 5 samples. The distribution of gB genotypes in transplant recipients group gB1 30.43%, gB2 39.13%, gB3 13.04%, gB4 17.39%, in congenital infection group gB1 66.67%, gB2 22.22%, gB3 11.11% and the other group gB1 70.59%, gB2 17.65%, gB3 5.88%, gB4 5.88%. No mixed genotype was found in any sample.

#### CONCLUSIONS

gB1 was the most frequent genotype in the patients included in this study but in transplant recipients group gB1 and gB2 had almost similar frequencies in non-transplant recipient group and the frequency of gB1 was significantly higher in this group compared to transplant recipients group (p=0.02). CMV gB is a target of the cellular immune system so different genotypes led to different immunopathological responses which leads to different disease manifestations this may be also related with the different distribution of gB genotypes in different patient groups. In addition, in our study, we detected different gB genotypes in different samples of one patient those were obtained during different episodes which suggest that reinfection may occur with different genotypes.

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**INTRACELLULAR PHARMACOKINETICS (PK) OF INTRAVENOUS (IV) AND ORAL BRINCIDOFOVIR (BCV)**M.B. Wire<sup>1</sup>, J. Dunn<sup>1</sup>, T. Arumugham<sup>1</sup>, M. Anderson<sup>1</sup>, M. Morrison<sup>1</sup>, O. Naderer<sup>1</sup><sup>1</sup>Chimerix, Durham, NC, USA**BACKGROUND-AIM**

BCV is a lipid conjugate nucleotide that has shown rapid viral clearance in patients with adenovirus infection and improved survival in animal models of smallpox following oral dosing. BCV is converted intracellularly to active cidofovir diphosphate (CDV-PP). We evaluated the peripheral blood mononuclear cell (PBMC) PK of CDV-PP following IV and oral suspension BCV administration.

**METHODS**

Two single dose BCV studies in healthy subjects contributed data for this analysis. Study CMX001-124 was a 2-period crossover bioequivalence study that enrolled 24 subjects who received two formulations of BCV oral suspension 100 mg fasted, one in each period. Data from 12 subjects who had PBMCs collected in Period 1 were included in this analysis; these subjects were 100% female, 31-59 years, and 58-88 kg. Study CMX001-123 enrolled 40 subjects who received IV BCV or placebo. Data from 18 subjects who received BCV 50 mg as either a 2-h or 4-h IV infusion were included in this analysis; these subjects were 100% male, 18-46 years, 64-106 kg. Serial PBMC samples were collected over 14 days and assayed by HPLC-MS-MS. PBMC CDV-PP PK parameters were determined by non-compartmental analysis.

**RESULTS**

Single doses of BCV 50 mg administered as 2- or 4-h IV infusions delivered intracellular CDV-PP exposures comparable to BCV 100 mg oral suspension. PBMC CDV-PP exposure was inversely related to infusion duration.

Geometric mean (GM) (coefficient of variation for GM; CVb%) intracellular CDV-PP AUClast (pg.h/106 cells) values were:

\*1107 (50%) for BCV 100 mg oral suspension

\*1409 (36%) for BCV 50 mg as 2-hr infusion

\*963 (36%) for BCV 50 mg as 4-hr infusion

GM (CVb%) intracellular CDV-PP Cmax (pg/106 cells) values were:

\*7.68 (41%) for BCV 100 mg oral suspension

\*14.3 (63%) for BCV 50 mg as 2-hr infusion

\*8.41 (57%) for BCV 50 mg as 4-hr infusion

**CONCLUSIONS**

IV BCV provided comparable PBMC CDV-PP exposures with lower doses when compared with oral BCV, which may allow for improvements in BCV tolerability and efficacy. The intracellular CDV-PP PK findings support evaluation of repeat dose IV BCV administration in healthy subjects and virally-infected patients.

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**QUANTIFICATION OF TORQUE TENO VIRUS AND EPSTEIN-BARR VIRUS FOR PREDICTING THE NET STATE OF IMMUNOSUPPRESSION AFTER LUNG TRANSPLANTATION: A PROSPECTIVE STUDY**R. Nordén<sup>1</sup>, J. Magnusson<sup>2</sup>, A. Lundin<sup>1</sup>, K. Tang<sup>1</sup>, M. Lindh<sup>1</sup>, L.M. Andersson<sup>1</sup>, G.C. Riise<sup>2</sup>, J. Westin<sup>1</sup><sup>1</sup>Department of Infectious Diseases/Clinical Virology, Institute of Biomedicine, University of Gothenburg<sup>2</sup>Department of Internal Medicine/Respiratory Medicine and Allergology, Institute of Medicine, University of Gothenburg**BACKGROUND-AIM**

The largest issues, early after lung transplantation are acute rejections and infectious complications. Adequate methods for monitoring immune suppression status are lacking. Here we evaluated torque teno virus (TTV) and Epstein-Barr virus (EBV) quantification as biomarkers for defining the net state of immunosuppression in lung transplanted patients.

**METHODS**

This prospective single centre study included 98 patients, followed for two years after transplantation. Bacterial infections, fungal infections, viral respiratory infections, cytomegalovirus viremia, and acute rejections as well as TTV and EBV levels were carefully monitored. In addition, the immunosuppressive regimen was logged for each patient and the levels of the respective drug was measured continuously in serum.

**RESULTS**

In our study group, post transplantation immunosuppressive treatments consisted of prednisone and mycophenolate mofetil that was completed with either cyclosporine (CSA) or tacrolimus (TAC). We found that the serum levels of TTV differed significantly between the groups that were treated with either CSA or TAC 6-24 months (p-value between 0.01 and 0.0006) after transplantation, indicating that serum levels of TTV DNA reflects a difference in immune status. In contrast, EBV levels did not differ between the two groups. However, contrary to previous published studies we did not find any correlation between serum levels of TTV or whole blood levels of EBV and infectious episodes at any time point post transplantation. No other confounding factors, that would explain the observed difference in TTV DNA-levels between TAC and CSA treated patients, were found. Also, neither TTV nor EBV levels were associated with episodes of acute rejection during follow-up.

**CONCLUSIONS**

In summary, TTV DNA levels in serum appear to reflect alterations in immune functions in lung transplanted patients but its use as a biomarker for defining the net state of immunosuppression in a clinical setting appear to be limited. Further studies are warranted to define differences in immune function as a result of TAC and CSA immunosuppressive treatment respectively.

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### CYTOMEGALOVIRUS DRUG RESISTANCE IN IMMUNOCOMPROMISED PATIENTS

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#### BACKGROUND-AIM

The study describes CMV drug resistance cases emerged in immunocompromised patients.

#### METHODS

From November 2014 to May 2017, CMV drug resistance was suspected in 19 immunocompromised patients for persistent CMV DNAemia levels after >2 weeks of antiviral treatment. In particular, 6 organ solid transplant recipients (liver n=3, heart n=2, multivisceral n=1) 12 hematopoietic stem cell transplant (HSCT) recipients and a patient with autoimmune diseases (Granulomatosis with Polyangiitis, GPA) were included in the study. In order to identify the most common mutations associated with CMV drug resistance the Sanger sequencing of amplified UL97 and UL54 gene segments was performed.

#### RESULTS

Mutations in CMV genome conferring drug resistance were found in 6 cases: 4 HSCT recipients, 1 multivisceral transplant recipient and 1 patient with GPA. A single mutation in UL97 gene conferring resistance (R) to ganciclovir (GCV) was detected in 3 HSC recipients: A594V (n=2) and L595S (n=1). The treatment was shifted to foscarnet (FOS) that led to undetectable CMV DNAemia in all cases. No patient developed CMV disease. In the remaining 3 cases the emergence of multidrug resistant CMV strains was documented. The onset of an initial single mutation (GCV-R) was identified in the UL97 gene after antiviral treatment: A594V in HSC recipient and in patient with GPA, M460V in the multivisceral transplant recipient. In these cases, the treatment was shifted to FOS followed by cidofovir (CDV). However, after a decrease, the CMV DNAemia levels increased again and additional mutations was found in the UL54 gene. For the HSCT recipient, the A834P mutation (GCV-R, FOS-R and CDV-R) was identified. The management of the patient is still under evaluation. For the multivisceral transplant recipient, the mutations Q578H (GCV-R, FOS-R, CDV-R) and E756D (FOS-R) were detected and antiviral treatment was stopped. The patient was managed with experimental therapy and infusion of HCMV immunoglobulin. Finally for the patient with GPA the mutation P522S (FOS-R) was detected. The patient developed a fatal CMV systemic reactivation.

#### CONCLUSIONS

Multidrug resistance is mostly associated with combined UL97/UL54-mutations. Drug resistance test is a tool to guide the choice of antiviral therapy.

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### HCMV- SPECIFIC ANTIBODY MEDIATED NK- CELL ACTIVATION AND NEUTRALIZATION IN LUNG TRANSPLANT RECIPIENTS

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#### BACKGROUND-AIM

Human Cytomegalovirus (HCMV) causes severe infections and increased mortality in lung-transplant recipients (LTR). The HCMV specific antibody (AB) response limits virus spread in LTR but its fine specificity is poorly understood. We analyzed the post-transplant development of HCMV neutralization and NK-cell activation in HCMV seropositive recipients (R+) and its relation to HCMV replication.

#### METHODS

Thirty-five seropositive LTRs were routinely followed up by HCMV-PCR and were retrospectively investigated for HCMV-specific neutralization (defined by NT50 in a neutralization ELISA) and NK-cell activation (defined by effective concentration EC50 in a bioluminescent reporter-assay). From each patient, plasma samples were obtained within the first week after transplantation, at stop of (Val-) Ganciclovir prophylaxis (three months post-transplantation), at start of viremia or in non-viremic persons at a similar time point (five months post-transplantation) and after one year after transplantation.

#### RESULTS

HCMV neutralization was lowest at the stop of (Val-) Ganciclovir prophylaxis and increased within one year post-transplantation to levels observed in healthy seropositive persons (both P=0.0001; Friedmann test). Clearly lower NT50 at three months post transplantation, were observed in patients with further HCMV replication (P=0.02, Mann-Whitney test) and a NT50 >54 was significantly associated with freedom from viremia (p=0.034; Fisher's exact test). Thereafter, at start of viremia, a NT50>425 was significantly associated with freedom from viremia (p=0.014; Fisher's exact test). HCMV-specific NK-cell activation was lowest at the stop of (Val-)Ganciclovir prophylaxis and increased within five months-transplantation to levels observed in healthy seropositive persons (P=0.0001; Friedmann test).

#### CONCLUSIONS

The level of HCMV-AB functions after lung-transplantation plays an important role in limitation of HCMV-replication in seropositive LTRs. Especially, the HCMV- specific neutralization was significantly higher in LTRs without viremia at stop of antiviral prophylaxis, which may potentially be useful to improve HCMV- management in R+ LTRs.

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### HEPATITIS E VIRUS-INFECTION IN ALLOGENIC HEMATOPOETIC STEM CELL TRANSPLANT RECIPIENTS

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#### BACKGROUND-AIM

Hepatitis E virus (HEV) is recognized as a cause of chronic infection in immunocompromised patients that may lead to cirrhosis and organ failure. HEV of genotype 3 is commonly found in wild animals in Sweden and the seroprevalence among healthy blood donors is around 17 %. At present, blood donors are not universally screened for HEV and transplant recipients may therefore be at risk of acquiring the virus.

#### METHODS

We screened serum samples from 262 allogeneic stem cell transplant recipients (241 adults and 21 children) taken 6 months after allogeneic hematopoietic stem cell transplantation (alloHSCT) for HEV RNA by RT-PCR and HEV IgG and IgM antibodies by ELISA (Dia.Pro). Samples positive for HEV RNA were amplified and Sanger-sequenced. Phylogenetic analysis was performed to establish type of HEV strain

#### RESULTS

We found that 39 patients (14.9 %) were IgG-positive for HEV, three (1.1 %) were IgM-positive and nine (3.4 %) had detectable levels of HEV RNA in serum at month six after alloHSCT. In total, 45 patients (17.2 %) were positive in at least one test and from which additional samples, donor and recipient, from before alloHSCT were obtained. In total, 40 out of 45 donor samples were available, only two were positive for anti-HEV antibodies and none had detectable levels of HEV RNA in serum. 39 of 45 pre-transplantation samples from recipients were available, nine were IgG-positive and two were positive for HEV RNA. The two patients who were RNA-positive before transplantation had detectable HEV RNA in consecutive follow-up samples (6 and 8 months respectively post alloHSCT) indicating chronic infection. Phylogenetic analysis revealed that three HEV-strains belonged to subtype 3II and one strain to 3IA. Subtype 3II is frequently found in wild and domesticated animals whereas 3IA have been found in solid organ transplant recipients and blood donors in Sweden. At this point we have not been able to definitely establish route of transmission, but the data indicate blood transfusion as the most likely event in all cases.

#### CONCLUSIONS

HEV is a rare cause of liver disease in alloHSCT recipients. Most patients that acquired HEV in this study cleared the infection during the follow-up period but 2 individuals (0.8 %) had signs of chronic hepatitis.

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### TEMPORAL DYNAMICS OF LUNG AND BLOOD VIROMES IN LUNG TRANSPLANT RECIPIENTS

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#### BACKGROUND-AIM

The human virome has an important influence on human health and disease, especially in immunocompromised individuals, such as lung transplant (LuTx) recipients. Viral communities differ through time in various niches of the human body, reflecting the need to study each compartment in detail. However, the virome dynamics in different body sites has not been thoroughly studied. Thus, we used shotgun metagenomics to characterize and compare the viromes of LuTx patients over time and in two body sites: lungs and blood.

#### METHODS

We sequenced 23 paired bronchoalveolar lavage (BAL) and blood samples from 7 LuTx patients taken at several time points post-transplantation on a MiSeq instrument (46 samples in total). After removing human and bacterial reads, the remaining sequences were aligned against viral genomes for taxonomic classification. The relative composition of viral families and strains over time and the Shannon diversity index between samples and between time points were assessed and sequencing results were compared with viral loads measured by qPCR.

#### RESULTS

We identified 10 viral families, including bacteriophages, in the BALs and in the blood samples. Torque Teno Viruses (TTV) of the Anelloviridae family were the most abundant in both body sites and were present in all patients. A higher TTV abundance (Wilcoxon rank sum test,  $p < 0.0001$ ) and Shannon diversity (Wilcoxon rank sum test,  $p < 0.01$ ) were observed in the blood. High variation in diversity over time was observed. We detected mixtures of the major TTV genogroups at unprecedented detail with up to 38 different strains detected within a single individual. These TTV populations varied between patients and time points. No significant difference was observed in the viral diversity according to preoperative diagnosis (Wilcoxon rank sum test,  $p > 0.05$ ).

#### CONCLUSIONS

The BAL and blood viromes of LuTx patients are mainly composed of anelloviruses. Our analyses revealed a broad spectrum of virus strains, especially of Torque Teno Viruses, coexisting within single patients. These populations, both in the BALs and in the blood, are very dynamic and change constantly over time. Some strains are persistent during all time points, while others occur only sporadically.

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### HEPARAN SULFATE GLYCOSAMINOGLYCAN, A KEY PLAYER FOR ENTEROVIRUS-71 DISSEMINATION IN HUMAN

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#### BACKGROUND-AIM

Enterovirus-71 (EV-71) causes hand, foot and mouth disease (HFMD), a mild and self limited illness, sometimes associated with severe neurological complications. EV-71 neurotropic determinants remain ill-defined. We identified a mutation (VP1L97R), acquired in the course of a disseminated infection in an immunocompromised host. The mutation was absent in the respiratory tract but present in the gut (as mixed population), blood and cerebrospinal fluid (as dominant species). This substitution was associated with a second substitution (VP1E167G) after amplification in cell culture (Cordey et al, 2012). Here we demonstrate that these mutations enable EV-71 to bind the ubiquitously expressed Heparan Sulfate Glucosaminoglycan (HSGAG) attachment receptor and change its tissue tropism.

#### METHODS

EV-71-VP197L167E (the respiratory variant) and EV-71-VP197R167G (the disseminated variant) were obtained by reverse genetics and their tropism and receptor usage was analyzed in cells and 3D reconstituted respiratory and gastrointestinal tissues. Binding and replication assays were performed in presence and absence of HSGAG. HSGAG expression levels were measured by immunofluorescence.

#### RESULTS

Removal of HSGAG from the cell surface affected binding and replication of EV-71-VP197R167G while EV-71-VP197L167E was not affected. In respiratory tissues, the replication of EV-71-VP197L167E was higher upon infection from the air-exposed interface, while EV-71-VP197R167G grew better after basal infection. Immunofluorescence showed that this correlated with the low and high expression levels of HSGAG at the apical and basal surface of the tissue, respectively. In the reconstituted intestinal tissues both variants replicated to similar extent.

#### CONCLUSIONS

We showed that the VP1L97R/E167G mutations acquired in the immunocompromised host conferred EV-71 higher binding affinity for HSGAG. Our data demonstrated that these mutations could appear after viral dissemination to the basal side of the respiratory tissue or the intestine tissue where the HSGAG binding variant was either as or more fit than the respiratory variant. Antivirals mimicking the HSGAG receptor may efficiently block EV-71 dissemination and neurotropism.

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### CLINICAL USEFULNESS OF IMMUNOLOGIC MONITORING OF CMV- SPECIFIC T-CELL RESPONSE IN LUNG TRANSPLANT RECIPIENTS

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#### BACKGROUND-AIM

Cytomegalovirus (CMV) remains one of the most common virus infection with significant morbidity and mortality in lung transplant recipients (LTRs).

This study aims:

- To analyze the clinical utility of monitoring CMV-specific T-cell responses in LTRs undergoing prophylaxis.
- To evaluate the kinetics of CD4+ and CD8+ T-cell counts and correlation between nadir CD4 T-cell count and infections after LT.

#### METHODS

A total of 21 patients underwent LT between February and June 2017, at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Thoracic Surgery and Lung Transplantation Unit, Milan, Italy.

Immunological monitoring involves evaluating CMV-specific T-cell responses in before transplant, and then regularly from the fifth week after transplant, once a month up to 24 months. At the Virology Unit, CMV-specific IgG in serum was measured by chemiluminescence immunoassay (CLIA) (DiaSorin SpA, VC, Italy) and CMV DNA levels were quantified by real time PCR (ELITech group, Turin, Italy).

We performed two interferon gamma (IFN-γ) release assays (IGRAs): QuantiFERON-CMV assay (Qiagen; Hilden, Germany) by stimulation of CD8+ T-cell responses and enzyme-linked immunosorbent spot (ELISPOT) assay (T.SPOT.CMV; Oxford Immunotec, Oxfordshire, UK) by stimulation of CD4+ and CD8+ T-cell responses. Normalization of ELISPOT responses to T-cell counts was used to assess both CD4+ and CD8+ T-cell responses.

CD4+ and CD8+ T cell counts were determined by flow cytometry.

#### RESULTS

Preliminary results have allowed to identify different groups of patients each with different immune response:

- Patients with stable levels of CMV specific CD4+ cells response, associated with lower risk of CMV replication.
- Patients with low levels of specific T-cell immunity, correlated with an increased risk of CMV disease and opportunistic infections.
- CMV-seronegative recipients of organs from CMV-seropositive donors (D+/R-) have a very high risk of developing primary CMV infection.

#### CONCLUSIONS

Our study demonstrate the relevant clinical of Immunological monitoring and to allow:

- The risk stratification and to personalize the CMV-specific T-cell response to arrive at a personalized therapy.
- Obtain experience for reduce the period of prophylaxis improving the clinical management of LTRs.

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# PROGRESSION OF BK POLYOMAVIRUS REPLICATION TOWARDS POLYOMAVIRUS ASSOCIATED NEPHROPATHY IN KIDNEY TRANSPLANT RECIPIENTS IS ASSOCIATED WITH AN INCREASE OF CHEMOKINE CXCL-10

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## BACKGROUND-AIM

The chemokine CXCL-10 has been proposed as inflammatory marker for disease severity and progression in diverse viral infections. In this study we assessed CXCL-10 levels in kidney transplant recipients (KTRs) in whom BK polyomavirus (BKPyV) detection in urine progressed to BKPyV DNAemia which finally resulted in polyomavirus associated nephropathy (PVAN).

## METHODS

The study included 160 serum samples from 72 KTRs who during the post-transplant follow-up showed at least one episode of BKPyV DNA detection in urine which was also associated with viral DNAemia (>103 copies/ml, detected by quantitative in-house PCR). Samples from 10 KTRs who did not display BKPyV detection during the post-transplant follow-up served as controls. CXCL-10 levels in serum were analyzed using quantitative ELISA.

## RESULTS

In parallel to the progression of BKPyV replication towards PVAN, we observed a stepwise increase of individual CXCL-10 levels. CXCL-10 first increased from baseline levels (prior to viral replication) when BKPyV DNA was detected only in urine ( $p=0.0005$ ). CXCL-10 levels furthermore increased when low and moderate BKPyV DNAemia (102-104 copies/ml) without the evidence for PVAN occurred ( $p=0.0001$ ). Finally, when high viral loads (>104 copies/ml) were detected and PVAN was diagnosed (detection of decoy cells and/or confirmation by histology), CXCL-10 levels peaked ( $p<0.0001$ , matched non-parametric t-test respectively). Interestingly, highest CXCL-10 serum levels were observed when, in addition to high-level BKPyV DNAemia and PVAN, KTRs displayed an episode of JC Polyomavirus ( $p=0.0059$ ) or Human Cytomegalovirus DNAemia ( $p<0.0001$ , Mann-Whitney test respectively) during the follow-up.

## CONCLUSIONS

Progression of HPyV replication in KTRs is associated with a stepwise increase of CXCL-10 levels, indicating that CXCL-10 could be used as a marker for PVAN development.

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# HIGHER ANTI-JCV ANTIBODY LEVELS ARE ASSOCIATED WITH TYPE O BLOOD GROUP

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## BACKGROUND-AIM

JC virus (JCV) associated progressive multifocal leukoencephalopathy (PML) occurs mostly in the context of severe impairment of the immune system. The level of the anti-JCV antibody response (so-called serum index values) are accepted risk factors for the later development of PML but the reasons for this are not well understood. Host genetic factors may also influence the anti-JCV antibody levels, and thus may be the underlying cause for such link. The blood group O has previously been shown to display a trend for an over-representation in cases with PML (Khoury et al, JAMA Neurology 2013). Aim of this study was to assess if type O blood group associated with higher levels of anti-JCV antibodies and could therefore be a risk factor for the development of PML.

## METHODS

We characterized ABO blood group antigen on blood samples of 62 patients with PML, and 64 controls without PML. Serum were tested in an enzyme-linked immunosorbent assay using a JCV-VP1 protein fused to GST as antigen, and anti-JCV antibody levels in arbitrary units (AU) were determined as previously published (Warnke et al, MSJ 2013).

## RESULTS

Of the patients with PML and known underlying disease, 62% were patients with MS treated with natalizumab, 14% were HIV positive, and 11% had underlying malignancy. JCV antibody levels were higher in MS patients with blood group O compared with all other blood groups, irrespective of the development of PML (O: median AU: 136; not O: median AU: 53;  $p<0.01$ ). This association was not observed for the closely related BK virus. Of the 62 patients with PML, 29 (47%, 95% CI 35-59%) were of blood group O, which showed a non-significant trend to differ from the expected distribution in the German population (41%), and the MS controls studied (23/64=36%, 95% CI 25-48%). In the natalizumab-associated PML subgroup, this deviation was the most prominent with 16 of the 29 cases having blood group O (55%, 95% CI 38-71%).

## CONCLUSIONS

Higher anti-JCV antibody levels are associated with the ABO blood group O antigen and may impact the risk of the later development of PML. The over-representation of blood group O in cases with PML was in line with a previous publication.

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# VERIFICATION OF QIAGEN ARTUS® BK VIRUS QS-RGQ KIT FOR DETECTION AND QUATIFICATION OF BKV DNA IN WHOLE BLOOD AND URINE SAMPLES

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## BACKGROUND-AIM

The objective of this study was to assess the performance of the real-time PCR Qiagen artus® BK Virus QS-RGQ kit on extracts from whole blood and urine samples extracted on QIAAsymphony. Results were compared to those received from reference laboratory and those expected from a serial dilution of the 1st WHO International Standard for BK Virus DNA.

## METHODS

The correlation, accuracy and reproducibility of the BKV quantitative and qualitative results between Qiagen artus® BK Virus QS-RGQ kit and the reference laboratory were compared. Forty two clinical samples were selected, including 25 whole blood samples (WB), with a viral load (VL) range of 1.70-5.75 log<sub>10</sub> copies/mL and 17 urine samples (U), with VL range of 2.16-9.73 log<sub>10</sub> copies/mL, including 36 positive samples. Testing was also performed on the 1st WHO International Standard for BK Virus DNA (NIBSC code: 14/212). Samples were extracted on the Qiasymphony SP using 'Virusblood200\_V5\_DSP default IC' protocol and DSP DNA Mini kit (Qiagen). The PCR was set up on Qiasymphony AS and run on Rotor-Gene Q thermal cycler.

## RESULTS

Intra-run and inter-run reproducibility were compared for both WB and U samples separately. Intra-run reproducibility was 88.2% and 89.5 for WB and U samples respectively. Inter-run reproducibility demonstrated 66.7% and 92.3% for WB and U samples respectively. Inter-run reproducibility of concentrations of the standards provided in artus® BK Virus QS-RGQ kit (concentrations: 10, 100, 1000 and 10,000 copies/μL) returned differences of less than 0.06 log<sub>10</sub> copies/μL. The mean value of the differences for WB samples was 0.74 log<sub>10</sub> copies/mL, and for U samples was 0.47 log<sub>10</sub> copies/mL. R<sup>2</sup> values of 0.92 achieved for WB samples and 0.99 for U samples demonstrating a good correlation between the two assays. Coefficients of variations for the standards included in the artus® BK Virus QS-RGQ kit ranged from 4.3 to 6.9 %. LODs were determined from linearity graphs and were 3.69 log<sub>10</sub> copies/mL and 2.69 log<sub>10</sub> copies/mL for WB and U samples respectively.

## CONCLUSIONS

Artus kit was not able to quantify low viral loads (below 3 log<sub>10</sub> copies/mL), but was able to detect the presence of BKV DNA. Intra-run and inter-run reproducibility and the achieved limits of detection were sufficient.

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# PARVOVIRUS B19 DNA POSITIVITY IN PATIENTS ATTENDED TO A UNIVERSITY HOSPITAL IN ANKARA, TURKEY IN 3 YEARS PROCESS

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## BACKGROUND-AIM

Human parvovirus B19 (B19) has been linked with a broad spectrum of clinical syndromes, including erythema infectiosum, transient aplastic crisis, persistent infection manifesting as pure red cell aplasia in immunocompromised individuals, nonimmune hydrops fetalis, arthritis, and rarely neurological manifestations. Generally, solid organs or stem cell recipients are at increased risk for viral reactivation. Parvovirus B19 DNA can be detected while serological tests are negative, even if the symptoms have not appeared yet. Therefore the use of real time PCR method is quite significant for early diagnosis of B19 infections. The aim of the present study is to investigate B19 DNA retrospectively, in samples that were sent to our laboratory.

## METHODS

The patients attended to Gazi University Hospital, between March 2014 to February 2017 were included in the study. Viral nucleic acids were extracted from the samples using MagNA Pure Compact Nucleic Acid Isolation Kit, in MagNA Pure Compact Instrument (Roche Diagnostics, Germany) device. Viral DNA amplified by Real Time PCR, using Light Mix® Parvovirus B19 Detection Kit (TIB Molbiol GmbH, Germany) that includes B19 primers and hybridization probes in Light Cycler 2.0 (Roche Diagnostics, Germany).

## RESULTS

Totally 211 specimens (193 blood, 8 CSF, 6 amnion fluid, 2 endotracheal aspirate, 1 bronchoalveolar lavage, 1 pleural effusion) were sent to our laboratory, from 189 patients between 0-92 years. Among these, 8% (17/211) of the samples that belong to 11 patients, were B19 DNA positive and 6 of them were female, 5 were male. All patients from neonatal intensive care unit that suffer from anemia, sepsis, hidrops fetalis or other neonatal disorders were negative for B19 DNA. A kidney transplant patient who had anemia, were positive with 108 copy/ml. Another patient who has acute lymphoblastic leucemia, had 106 copy/ml. The other 9 positive patients had viral loads between 101-105 copy/ml who were from haematology, nephrology, oncology and paediatric clinics and had anemia/aplastic anemia, transplantation or malignity.

## CONCLUSIONS

Detection of B19V in a short time in immunosuppressed patients by Real Time PCR is an advantageous method. Therefore, determination of B19 DNA in these group of patients is important for early diagnosis.

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### **SIGNIFICATION OF DNA DETECTION OF PATHOGENS IN BIOPSY TISSUES IN PATIENTS AFTER TRANSPLANTATION OF HEMATOPOIETIC CELLS**

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#### **BACKGROUND-AIM**

The incidence of infectious complications in patients after hematopoietic cell transplantation is one of the major limiting factors of good prognosis for successful treatment of hematologic malignancies. Due to targeted immunosuppression under the transplantation procedure, repeated and often life threatening reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV), Human herpes virus 6 (HHV 6), parvovirus B19 (PB19) and adenovirus (ADV) occur particularly in these patients. Regular monitoring for the presence of viral DNA is the therapeutic standard of care in patients after hematopoietic cell transplantation.

#### **METHODS**

The whole blood samples are routinely collected in K3EDTA tubes. Recently, the detection of viral DNA in samples of biopsy tissue is increasingly required in the differential diagnosis. This is due to confirm organ disease. Sets RealStar PCR kit (Altona) and the detection system CFX 96 (Biorad) were used for the amplification of each pathogen. Tissue isolation was performed by commercial isolation kit QIAamp DNA Mini kit (Qiagen) using a tissue protocol.

#### **RESULTS**

We retrospectively investigated a group of 92 patients. Total of 156 biopsy tissues samples were collected to detect viral DNA (CMV, EBV, HHV-6, PB19, ADV) in the years 2012 - 2016. Most tissues were taken from the gastrointestinal tract (53%), skin (40%), others (7%). The presence of at least one pathogen was confirmed in 82% of the samples. In relation to postransplantation prognosis, the absence of relapse of the underlying disease was associated with more frequent HHV6 and PB19 in tissue samples (81% vs. 44%  $p = 0.04$  and 67% vs. 22%  $p = 0.03$ ). Higher probability of death was associated with more frequent detection of HHV6 in GIT (93% vs 59  $p = 0.04$ ).

#### **CONCLUSIONS**

In some cases, the diagnosis of post-transplant complications is very difficult. Clinical picture of patient can be very variable, confirmation of the absence of viral DNA in the blood does not often correlate with the clinical data. Biopsy tissue sampling appears entirely relevant in the differential diagnosis of complicated post-transplantation conditions. It refers to a viral infection at a stage where there is organ viral replication without the release of viral particles into the blood.

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### **HUMAN HERPESVIRUS (HHV) SEROPREVALENCES WITH FOCUS ON HERPES SIMPLEX VIRUS (HSV): A 5-YEAR HOSPITAL-BASED STUDY**

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#### **BACKGROUND-AIM**

After primary infection, human herpesviruses (HHVs) persist in the host for life and may reactivate from latency with manifestations varying from asymptomatic shedding to serious clinical forms, especially in immunocompromised individuals. No recent study reports the current distribution of HHVs in the French population. In this context, the aim of our work was to study the trends of HHV seroprevalences with particular focus on herpes simplex virus (HSV) over 5 consecutive years (from January 2012 to December 2016).

#### **METHODS**

This study was based on the data collection of routine detection of HHV-specific IgG antibodies in sera recovered from patients hospitalized in La Pitié-Salpêtrière University Hospital, a major tertiary care centre in Paris, France.

#### **RESULTS**

Except for herpes simplex virus type 2 (HSV-2) with 21.9% of positive antibody detection, relatively high HHV seroprevalences were observed: 79.5% for herpes simplex virus type 1 (HSV-1), 95.4% for varicella-zoster virus (VZV), 72.3% for cytomegalovirus (CMV), and 96.3% for Epstein-Barr virus (EBV). HSV-2 seroprevalence was significantly higher in females than in males (31.6% vs 22.0%,  $p < 0.0001$ ). Interestingly, approximately 80% of patients with HSV-2 IgG positive detection were co-infected with HSV-1. The odds of having antibodies against CMV, EBV or human immunodeficiency virus (HIV) were significantly higher in HSV seropositive than HSV seronegative patients (for CMV: 79.4% vs. 46.1%,  $p < 0.0001$ ; for EBV: 98.1% vs. 90.8%,  $p < 0.0001$ , and for HIV: 2.2% vs. 0.6%,  $p = 0.0020$ ). Additionally, HSV, VZV, CMV, and EBV concomitant positive signals were observed in almost 50% of patients for whom a complete HHV serological testing panel was performed.

#### **CONCLUSIONS**

Our findings raise greater awareness among clinicians involved in care of patients at high risk for HHV-related diseases.

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### GENOTYPIC ANALYSIS OF GANCICLOVIR (GCV) RESISTANCE IN CYTOMEGALOVIRUS (CMV) ISOLATES

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#### BACKGROUND-AIM

Ganciclovir-resistant Cytomegalovirus (CMV) strains is reported following long term antiviral agent use especially for immune-suppressive patients. The aim of the present study is genotypic analysis of mutations on UL97 gene of CMV which causes ganciclovir (GCV) resistance in the patients who develop CMV infection following hematopoietic cell transplantation (HCT) or solid organ transplantation (SOT).

#### METHODS

Thirty patients who had HCT or SOT and developed CMV infection during routine follow-up with a viral load of CMV over 1000 copies/mL in Akdeniz University Hospital between June 2014 and March 2016 were enrolled into the study. CMV DNA was analyzed by an automated system (Cobas Ampliprep/COBAS Taqman CMV Test, Roche Diagnostics, Germany) quantitatively. DNA sequence analysis of the regions including codons 420-664 in UL97 gene region was done in order to detect mutations causing antiviral resistance and were compared with defined mutations.

#### RESULTS

Thirty patients enrolled include 22 patients who had HCT and eight patients who had SOT (five kidneys, three liver). When CMV serology pattern of the patients was evaluated before transplantation, 29 (96.7%) patients were found sero-positive whereas one (3.3%) patient was found sero-negative. Totally nine CMV UL97 mutations were detected in seven (23.3%) pediatric patients who had HCT including six sero-positive and one sero-negative cases. Five of the mutations defined are UL97 mutations with a defined clinical resistance against GCV in each of five recipients (C603W, C592G, H520Q, M460V, A594T). One mutation (D605E) which is known with an insignificant effect on UL97 function and does not cause GCV resistance was detected in one recipient whereas three mutations (I474T, F499S, V559A) which was not defined before in one recipient.

#### CONCLUSIONS

Clinical resistance-associated CMV UL97 mutation was detected in five (22.7%) of 22 patients who had HCT. CMV sero-positive recipient status of these patients increases risk of CMV infection and long term antiviral use increases ganciclovir resistance risk. Association of three different and new undefined mutations which was detected in one recipient should be searched with phenotypic tests.

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### COMPARISON BETWEEN WHO AND ACROMETRIX™ PANELS AND PERFORMANCES OF NEW VERSION OF BK VIRUS R-GENE® REAL TIME PCR ASSAY

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#### BACKGROUND-AIM

BK virus viral load is used to follow transplant patients to adapt the immunosuppressive treatment and avoid transplant rejection. Monitoring is usually done on urine and plasma or whole blood. Testing whole blood is of interest as laboratories may manage one unique workflow for all transplant viruses on a single specimen.

The first WHO International Standard for BK virus has been commercialized since 2016 and allows standardization compared to secondary standards. BK Virus R-gene® kit quantifies the BK virus in whole blood, plasma and urine. The new version of this kit, improving the interpretation on whole blood, has been tested on 2 standards.

#### METHODS

Determination of analytical sensitivity was performed on 3 matrices using the first WHO International Standard for BK virus and AcroMetrix™ BKV panel.

Comparison study between the previous and new version of BK Virus R-gene® kits on at least 100 positive clinical samples for the 3 matrices was performed.

#### RESULTS

With WHO, the LoD is:

- 2.5 log cp/mL for urine
- 2.3 log cp/mL for plasma
- 2.6 log cp/mL for whole blood

With AcroMetrix™, the LoD is:

- 2.1 log cp/mL for urine
- 1.8 log cp/mL for plasma
- 2.4 log cp/mL for whole blood

Comparison on 103 plasma and 117 urine positive samples indicates a perfect concordance on detection between the 2 versions of the kit and on the quantification with an average of differences of 0.0 (SD=0.2) log cp/mL.

Out of 101 positive whole blood samples, the new kit detects 33 additional low positive samples with viral load below 3.3 log cp/mL. Higher quantification (1.6 log cp/mL in average; SD=0.9) is observed with the new kit version on 19 samples with viral load from 3.1 to 4.0 log cp/mL.

#### CONCLUSIONS

Sensitivity determined with WHO and AcroMetrix™ panels varies from 0.2 to 0.5 log cp/mL depending on the matrix. Previous publications have described that multiple subpopulations on T region in WHO could induce a difference of viral quantification of up to 4-fold. The BK Virus R-gene® kit targets the incriminated area which could explain the observed difference.

The clinical samples study with previous and new version of the kit shows a preservation of performances on plasma and urine matrices while improving sensitivity on whole blood matrix.

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### PERFORMANCE OF ARGENE® TRANSPLANT ASSAYS ON EMAG®

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#### BACKGROUND-AIM

Management of immunosuppressed patients through molecular monitoring is of paramount importance and sample preparation remains key. ARGENE® Transplant panel has been designed to answer those needs with assays that are already validated with the NucliSENS® easyMAG® extraction platform. We present additional results that allowed their validation on eMAG®, as a complement to results previously shown on the equivalence of systems.

#### METHODS

Studies were designed to compare performance obtained on eMAG® with the ones already validated on NucliSENS® easyMAG®. Limits of Detection were tested for confirmation by testing a minimum of 20 replicates on the following targets: CMV in both whole blood and amniotic fluid, HSV1 and VZV in Cerebrospinal fluid. Clinical positive samples were also tested on both systems: CMV in amniotic fluid, Parvovirus B19 in medullary plasma, ADV in plasma and stool samples with various concentrations of target.

#### RESULTS

LoD were confirmed for all tested combinations (of target and specimen types), Ct value mean and standard deviation are indicated for each parameter: CMV in Whole Blood (21/21 positive detected; 36.6Ct & 0.8), CMV in Amniotic Fluid (20/20 positive detected; 35.6Ct & 0.5), HSV1 & VZV in CSF (21/21 positive detected each; respectively 37.3Ct & 0.7 and 37.7Ct & 1.5).

For studies on clinical samples, all results were in agreement, considering an acceptance criteria of  $\pm 0.5 \log_{10}$  cp/mL between the quantitative values obtained with both systems: 19/19 for ADV in plasma (inputs from 4.26 to 8.49  $\log_{10}$  cp/mL), 14/14 for Parvovirus B19 in medullary plasma (inputs from 2.15 to 8.70  $\log_{10}$  cp/mL), 8/9 for CMV in Amniotic Fluid (inputs from 1.92 to 5.57  $\log_{10}$  cp/mL) with 1 sample detected on eMAG® only with a Ct value >39, and finally 22/22 for ADV in stools (inputs from 22.7 to 41.5 Ct).

#### CONCLUSIONS

Confirmation of LoD and equivalence on clinical samples allowed, in addition to studies having shown the equivalence between extraction systems, to validate the bioMérieux ARGENE® Transplant kits in the various claimed matrices (whole blood, medullary plasma, CSF, amniotic fluid, stool, ...) on the eMAG® system. In the current environment of laboratory accreditation and related needs, proof of evidence through comparison of performance is of interest.

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### EVALUATION OF WHOLE BLOOD SPECIMEN HANDLING WITH FOUR BLOOD COLLECTION TUBE TYPES ON THE BECKMAN COULTER DXN VERIS MOLECULAR DIAGNOSTICS SYSTEM USING THE VERIS CMV ASSAY

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#### BACKGROUND-AIM

Accurate quantification of molecular diagnostic viral load assays are essential to the management of infected patients. Preanalytical parameters can contribute to variability in specimen stability and present a risk to patient safety for in vitro diagnostic viral load medical devices. Blood collection tubes (BCT) are a factor in the preanalytical process. BCT elements, such as anticoagulants and storage, can affect the accuracy of downstream molecular diagnostic assays. The objective of this study was to evaluate the VERIS CMV assay performance with respect to different types of BCTs after storage test conditions.

#### METHODS

Twenty donors donated 7 tubes of a single BCT type to generate a total of 20 replicates per test condition. The 4 BCT types tested were: BD K2EDTA, BD PPT-Gel, Sarstedt K2EDTA Plasma-Gel, and Sarstedt K3EDTA. CMV virus was spiked to each BCT at a final concentration of 600 IU/mL. One BCT was processed as the control, while the other six BCTs were equally split between 2 storage conditions, 30°C and 2°C, for 25 hours before plasma separation. After centrifugation, plasma from one BCT from each storage condition was transferred to an aliquot tube for assaying, a second BCT was assayed in the BCT, and a third BCT was placed at 34°C for 7 hours to simulate instrument onboard stability before assaying in the BCT.

#### RESULTS

To test primary BCT and aliquot tube equivalence, the quantified viral load was analyzed using matched pair analysis. There were no significant differences in mean  $\log_{10}$  IU/mL results between primary and aliquot tubes across all test conditions. To test whole blood specimen stability, mean viral load results from blood specimens in storage conditions were compared to fresh blood specimens. For all test conditions, the mean differences were  $<0.3 \log_{10}$  IU/mL with 95% confidence. Tube type equivalence was tested comparing the mean viral load results from blood specimens from the 4 different BCT types within each test condition. There were no significant differences in viral load results between BCT types within test conditions using 0.3  $\log_{10}$  IU/mL threshold with 95% confidence.

#### CONCLUSIONS

The VERIS CMV Assay showed equivalency between primary BCT and aliquot tubes, different whole blood storage conditions compared to fresh specimens, and between different BCT types.

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# PERFORMANCE EVALUATION OF COBAS® CMV FOR USE ON THE COBAS® 4800 SYSTEM: A NEXT GENERATION CMV ASSAY FOR STANDARDIZED CMV VIRAL LOAD MONITORING

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## BACKGROUND-AIM

Current cytomegalovirus (CMV) viral load (VL) testing is complicated by the heterogeneous landscape of in vitro diagnostic and lab developed tests. Standardization is important to ensure accurate and comparable results for patients across different laboratories and tests. The objective of this study was to evaluate the performance of the cobas® CMV for use on the cobas® 4800 System, a new automated CMV VL in vitro diagnostic assay.

## METHODS

The limit of detection (LOD) and accuracy of cobas® CMV was determined using a dilution panel made from the 1st WHO International Standard for CMV. The LOD was further verified for glycoprotein B genotypes 2-4 as well as CMV strains resistant to ganciclovir, valganciclovir, cidofovir, and foscarnet. Assay linearity was determined for glycoprotein B genotype 1 and also verified across genotypes 2-4 and drug resistant strains. Assay precision was also determined for glycoprotein B genotype 1, and specificity was examined in both CMV-negative samples and in the presence of potential interferents. A method correlation study was done on clinical and contrived samples comparing cobas® CMV on the cobas® 4800 System to the CE-marked cobas® CMV on the cobas® 6800 System.

## RESULTS

The limit of detection for cobas® CMV was verified for CMV glycoprotein B genotypes 1-4 and all tested drug resistant strains as 34.5 IU/mL, with a linear range up to 1E7 IU/mL. The assay was precise (SD <0.2 log10 IU/mL across the linear range) and traceable to the WHO Standard. Specificity was 100% in 611 CMV-negative samples, with no interfering substances or cross-reactivity with other pathogens seen. In 205 CMV samples tested, cobas® CMV on the cobas® 4800 System showed a mean difference of 0.1 log10 IU/mL to cobas® CMV on the cobas® 6800 System.

## CONCLUSIONS

cobas® CMV on the cobas® 4800 System demonstrated excellent sensitivity, specificity, and correlation to an existing state-of-the-art CMV assay. The assay is standardized to the WHO standard for CMV, thereby providing results in IU/mL. Inclusion on the automated cobas® 4800 System also allows for mixed batching with other virology assays, helping streamline lab workflows and providing actionable and timely results for patients with CMV infection.

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# STANDARDISATION OF HERPES SIMPLEX VIRUS DNA NUCLEIC ACID TESTS

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## BACKGROUND-AIM

Herpes simplex virus type-1 and 2 (HSV-1, HSV-2) have a high global prevalence, causing facial lesions (predominately caused by HSV-1) and genital ulcers (predominately caused by HSV-2). HSV is an important pathogen of the central nervous system (CNS) manifesting either as meningitis (HSVM) or encephalitis (HSVE). Untreated HSVE can lead to focal necrotizing encephalitis which has high mortality rates in the region of 70%.

## METHODS

The use of molecular techniques for the diagnosis of patient infection is recognised as the reference method, predominately the specific diagnosis of CNS infections. Early and accurate diagnosis is therefore essential in the treatment of viral CNS infections. Accurate diagnostics is especially important in immunocompromised patients who may present with atypical lesions and may be slow to respond to therapy.

## RESULTS

Many diagnostic assays allow for the simultaneous detection of HSV-1 and 2. However the majority of assays report results in a qualitative format and have been shown to vary greatly in sensitivity. A recent study conducted by NIBSC where a lyophilized multiplex panel comprising HSV1 and HSV2 was sent to 17 laboratories to assess for HSV1 and 16 laboratories to assess HSV2 using their routine NAT assay. The data returned showed variation in measurement >3.5 Log10 Copies /mL for both HSV1 and HSV2. There is a need for an international standard(s) to allow the accurate determination of assay sensitivity for both HSV-1 and HSV-2 in order to reduce variation in measurements from different laboratories and therefore ensure correct and timely patient treatment management.

## CONCLUSIONS

At NIBSC two lyophilised candidate standard reference materials comprising whole virus laboratory cultured strains of HSV 1 and 2, formulated in universal buffer (10mM Tris buffer, 0.5 % human serum albumin, 0.1% trehalose) with a concentration ~ 1x10<sup>7</sup> genomes/mL, have been prepared. An international collaborative study is scheduled for October 2017 to evaluate their suitability to be established as the 1st WHO International standards for HSV-1 and HSV2 and assign potency in International unit (IU). This study will also include patient samples to assess commutability. We are looking for participants from clinical and reference laboratories as well as IVD manufacturers are invited to take part.

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**HTLV I-II POSITIVE SEROLOGY IN ONCOHEMATOLOGIC PATIENTS**S. Ranno<sup>1</sup>, L. Coltella<sup>1</sup>, G. Pizzichemi<sup>1</sup>, M. Onori<sup>1</sup>, C. Concato<sup>1</sup><sup>1</sup>Unit of Microbiology Virology and Parasitology, Laboratory Department, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy**BACKGROUND-AIM**

The human T-lymphotropic viruses (HTLV I-II) are human oncogenic retrovirus, associated with T-cell leukemia and lymphoma (HTLV-I) and rare neurological disorders (HTLV-II). Transmission is mother to child, sexual, and by contaminated blood products. Infections associated with blood transfusions or organ transplants are now rare, because, when risk factors such as birth in endemic countries or hazardous behaviors are present, donors are tested for HTLV I-II antibodies. Particularly, in bone marrow or hematopoietic stem cells transplantation, donor anamnesis and clinical or laboratory evidence for virus transmission, constitute exclusion criteria. As, recipient positivity for HTLV I-II, confirmed by proviral DNA recovery, compromises the recruitment for transplantation.

**METHODS**

We perform HTLV I-II antibody test by CMIA technology. A value  $S/CO < 1$  is related to a not reactive sample, while a value  $S/CO \geq 1$  indicates a positive sample. From March 2015 to September 2016, we tested 564 serum samples collected from 381 patients/donors belonging to the Oncohematology Unit. The same test was also performed on a batch of Immunoglobulin (Flebogamma DIF 50 mg/ml, Grifols), used for support therapy.

**RESULTS**

Of the 564 tested samples, 11% (61/564) was positive for HTLV I/II IgG with an average value of 2.02 (range 1.03-5.35  $S/CO$ ). On February 2016, this percentage raised to 34% (12/35). The test performed on Flebogamma resulted positive (Index 8.45  $S/CO$ ).

**CONCLUSIONS**

On February 2016 there was an unexpected number of positive samples, with values close to the cut-off (range 1.05-3.34), letting us to suppose an interfering factor. Considering that positive samples referred to patients and not to donors, and considering their negative history for risk factors infection, we hypothesized that positivity was associated with the presence of IgG anti HTLV I-II in the Immunoglobulin batch used for support therapy. Confirmation resulted from test performed on a non-diluted drug rate, which gave a straight positive result.

Thence, anti-HTLV I-II IgG positivity is not necessarily sign of infection. Testing the batch of immunoglobulin used, may be useful for evaluating both seroconversion not supported by anamnestic data and rapid disappearance of specific anti-HTLV I-II antibodies, otherwise unexplained.

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**MULTI-CENTRE STUDY: 2-YEAR EVALUATION OF ASSAY REPRODUCIBILITY FOR FOUR TRANSPLANT ASSOCIATED VIRUSES**A. Ricketts<sup>1</sup>, F. Opdam<sup>1</sup>, C. Netti<sup>1</sup><sup>1</sup>Qnostics, Glasgow**BACKGROUND-AIM**

Reproducible viral load determination plays a critical role in clinical diagnostics and a central role in monitoring patients' response to treatment and disease progression. A rolling 2-year multi-centre study is being run to assess the ability of clinical diagnostics providers to provide consistent results across a number of years. In the study four viral targets were assessed over multiple sites and platforms. We examined assay reproducibility and accuracy by comparing how different platforms quantified duplicate samples across four test cycles.

**METHODS**

Control materials for each of the 4 viral targets (Cytomegalovirus (CMV), Epstein Barr Virus (EBV), JC Virus (JCV) and BK virus (BKV)) were prepared at a single titre in human plasma at a concentration that falls within the linear range for most assays in use. The controls were characterised internally using both an in house qPCR based method and digital PCR (BioRad qX200 droplet ddPCR). Blind panels containing duplicate or triplicate samples were provided to laboratories participating in the study. Laboratories were asked to treat the materials as they would a clinical sample and to return quantitative data along with information on the assay workflow used to generate the result.

**RESULTS**

Data were anonymised, collated, and non-compliant results removed. The data were split into categories based on the assay manufacturer who provided the amplification reagents. Outliers were assessed through the application of Grubbs' analysis to each assay manufacturing group. Datasets were considered suitable for inclusion if more than one laboratory reported data using the same assay manufacturer after removal of non-compliant data and outliers. Assay manufacturing group datasets were compared against the group consensus and the dPCR assigned values.

**CONCLUSIONS**

The use of international standards and certified reference materials to align assay performance is essential if data transferability is to be achieved. In comparing viral targets that have a WHO international standard against two that have only just had a standard released we saw that the dPCR values were closely aligned with the consensus values of the study.

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### **VALSPODAR IS A NOVEL ANTI-CMV DRUG THAT LIMITS EARLY STAGES OF CMV GENE EXPRESSION AND DISSEMINATION**

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#### **BACKGROUND-AIM**

Human cytomegalovirus (HCMV) is a ubiquitous pathogen that establishes a life-long infection affecting 40-80% of the US population. CMV periodically reactivates leading to enhanced morbidity and mortality in immunosuppressed patients causing a range of complications including organ transplant failure and cognitive disorders in neonates. Therapeutic options are limited to a handful of antivirals that only target CMV replication causing the circulation of viral resistant strains. Additionally, immunocompromised patients, pregnant women, and infants do not tolerate these antivirals well signifying a need to develop novel therapeutics that target different steps of the viral life cycle with lower toxicity to patients. To this end, we screened a small molecule library that identified a 2nd generation p-glycoprotein (Pgp) inhibitor, Valspodar, with antiviral properties targeting steps prior to immediate-early gene expression following a virus infection.

#### **METHODS**

We performed a high-content screen using the AD169IE2-YFP-fibroblast infection model against the MedChem Express library of 123 compounds targeting membrane transporters and ion channels. The most effective compounds that inhibited a virus infection were Ca<sup>2+</sup> channel inhibitors and valspodar, a Pgp inhibitor. Given the novel function of Pgp in a CMV infection, we performed standard cell cytotoxicity assays and virology assays to further characterize the role of valspodar and Pgp.

#### **RESULTS**

Valspodar has no effect on virus entry into host cells but does limit immediate early gene transcription and translation. Unlike treatment with 1st or 3rd generation Pgp-inhibitors, cells can withstand high concentrations of Valspodar over a 7-day period with minimal cytotoxicity. Valspodar limits CMV plaque number and size in comparison to DMSO control assays demonstrating its ability to limit cell-to-cell spread of the virus. Lastly, cells that have received an siRNA targeting the Pgp ABCB1 are less supportive of virus replication concluding that this cellular factor plays a critical, yet unknown role in the CMV replication cycle.

#### **CONCLUSIONS**

Valspodar represents a novel anti-CMV therapeutic that limits CMV infection by way of a host cell target.

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### **CONVERSION OF TAQMAN BASED LDTs AS WELL AS QUANTITATIVE LDTs ONTO THE SAMPLE-TO-ANSWER ARIES PLATFORM**

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#### **BACKGROUND-AIM**

ARIES® is molecular, sample-to-answer system that combines nucleic acid extraction from clinical samples and real-time PCR detection in a closed, self-contained cassette. It is able to process 1 to 12 cassettes simultaneously and needs 5–10 min hands-on-time per 12 samples. Its characteristics makes it ideally suited for situations (1) where less trained personnel is available, e.g. during outside office hours or for molecular unexperienced labs, and/or (2) for point of impact testing where a quick result is needed to guide clinical decision making. Uniquely, ARIES has the run LDTs (lab developed tests) next to IVD tests. Up to now only qualitative LDTs were developed based on MultiCode, which is a probe-less real time PCR technology that leads to a decrease (i.e. quenching) in fluorescence. In this study ARIES data are shown of quantitative LDTs as well as LDTs utilizing the non-quenching real-time PCR technology (e.g. TaqMan and SYBR Green).

#### **METHODS**

Quantitative MultiCode-based and commercially available Luminex analyte specific reagents (ASRs) for BK, EBV, and CMV were used on ARIES. Analysis was performed using Luminex SYNCT software and, in some cases, Microsoft Excel. For quantification experiments, titrated material in a plasma matrix (Exact Diagnostics) was tested to develop a standard curve and assess inter-run error. For accessing the ability to develop a non-quenching LDT, a known and working Bordetella pertussis TaqMan assay was converted to the ARIES format and tested on a number of control samples. For SYBR Green an existing RT-PCR assay that detects bacterial RNA was used and put on ARIES.

#### **RESULTS**

When evaluating quantitative LDTs for BK, EBV and CMV on ARIES, similar Ct's to conventional (i.e. non-ARIES) procedures were observed. Inter-run error was determined to be <0.5 log when standards were run in triplicate. Positive and negative controls for the ARIES Bordetella pertussis TaqMan and bacterial SYBR Green LDTs gave expected results.

#### **CONCLUSIONS**

Proof of concept was shown for converting high complexity quantitative and qualitative lab developed test to a lower complexity sample-to-answer format on the Luminex ARIES system. Future efforts will be made towards a more in-depth sensitivity assessment and analysis software enhancements.

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# **HUMAN CYTOMEGALOVIRUS GANCICLOVIR TREATMENT FAILURE AND UL97 PHOSPHOTRANSFERASE RESISTANT MUTATION IN ADULT ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS**

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## **BACKGROUND-AIM**

To determine the incidence of ganciclovir-resistant cytomegalovirus (CMV) in adult hematopoietic stem cell transplant (HSCT) recipients in Hradec Kralove University Hospital Hematology Centre. CMV treatment failure (clinical resistance) was defined as a stable or increasing viral load in blood after 2 weeks of well conducted virostatic treatment. The association between the treatment failure and viral resistance was analysed. Presence of ganciclovir-resistant CMV strains was confirmed by genotypic testing.

## **METHODS**

58 patients after allogeneic HSCT for haematology malignancies treated for CMV reactivation/disease were prospectively followed from 2012 to 2014. In patients with treatment failure, CMV DNA was extracted and analysed by nucleotide sequence analysis of the UL97 and UL54 regions. CMV viremia was determined by a quantitative real-time PCR using artus CMV RG PCR Kit (Qiagen, Germany) from full peripheral blood sampled in tubes with EDTA. For DNA extraction QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used. The quantification cut off was 500 cp/ml and the detection cut off limit 100 cp/ml. CMV DNA samples were stored at -200C for retrospective sequence analysis of mutation development time. The statistical analyses were performed using SigmaPlot for Windows, version 11.0. (Systat Software), descriptive statistics were summarized for all patients.

## **RESULTS**

CMV reactivations were detected in 69% patients, more often in recipient serostatus D-/R+ or D+/R+ . The treatment failure occurred in 9 patients (i. e. 15.5% of patients receiving anti-CMV therapy) but ganciclovir (GCV) resistance coding mutations were proved only in 3 of them (mutations L595F, M460I, A594V in UL97). Single UL97 GCV resistance mutation was detected in each patient, no UL54 mutation. The median time of treatment failure diagnosis was 70 days after HSCT. Patients with treatment failure were treated with GCV for a longer cumulative time than the patients susceptible to administrated GCV (p = 0.003) and had a higher viral load peak (p = 0.005). The viral load at the start of maintenance therapy was also higher (p = 0.0173) and CMV disease occurred more often (p = 0.0152). Median time to treatment failure in 3 patients with proven resistant CMV was 168 days post HSCT (median 90 days of cumulative treatment) vs. 58 days in 6 patients without detected CMV mutation (median 16.5 days of cumulative VGCV/GCV treatment).

## **CONCLUSIONS**

Our data indicate that only a part of virostatic treatment failure cases is linked to viral resistant mutations. However, patients

with prolonged CMV replication, administered virostatic therapy and high or stable viral load should be monitored regularly and more detailed together with analysis of GCV resistance genes.

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# A PROSPECTIVE OBSERVATIONAL STUDY ON THE ORIGIN AND PATTERN OF HUMAN POLYOMAVIRUSES REPLICATION AFTER KIDNEY TRANSPLANTATION

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## BACKGROUND-AIM

Human Polyomaviruses (HPyVs) are ubiquitous DNA viruses establishing latent infections in the host. Immunosuppression is a recognized risk factor for HPyVs reactivation. The most extensively studied HPyV in kidney transplantation (KTx) is BK virus (BKV) but other HPyVs such as JC virus (JCV), Merkel Cell PyV (MCPyV), and Polyomavirus 9 (HPyV9) have been detected in KTx. Origin, natural history, and clinical significance of these HPyVs remain unclear.

## METHODS

Urine and blood samples from 27 KTx donor/recipient pairs were collected immediately before KTx and on post KTx day 1 (T1), 15 (T2), 30 (T3), 60 (T4), 90 (T5). Samples were tested for BKV, JCV, MCPyV, Polyomavirus 7 (HPyV7), and HPyV9 genome by virus-specific DNA duplex TaqMan Real Time PCR. Molecular characterization of the amplified viral strains was conducted by automated sequencing.

## RESULTS

No HPyVs viremia was observed whereas HPyVs viruria was detected in 12/27 (44.4%) donors and 13/27 (48.1%) recipients. Identical HPyV strains were isolated in 7/27 (25.9%) donor/recipient pairs. JCV genome was amplified in 11 donors and in 7 recipients since T1. JCV strains detected in the recipients were identical to those amplified in the paired donors. MCPyV DNA was detected in 4 recipients at T2. BKV genome was detected in 3 recipients from T3. Three recipients experienced concomitant replication of JCV and MCPyV. No relationship between HPyV replication and clinical course was identified during the first 3 months of follow up.

## CONCLUSIONS

Our data confirm that replicating JCV is frequently observed in organ donors and that JCV replication is common in the early post KTx phase. We demonstrated that JCV early post KTx infections are caused by viral strains transmitted by the donors. MCPyV and BKV post-transplant replications observed in this series were likely due to reactivation of recipient strains. Extended follow up is needed to rule out clinical impact of early JCV infection after KTx.

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# INVESTIGATION OF BKV AND JCV POSITIVITY IN PAEDIATRIC PATIENTS WITH KIDNEY TRANSPLANT

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## BACKGROUND-AIM

BK virus (BKV) is a causal agent of nephropathy and hemorrhagic cystitis in kidney transplant recipients, and is considered an important emerging disease, with the risk of kidney rejection, in transplantation. The purpose of this study is investigation of BKV and JCV DNA in paediatric patients with kidney transplant and evaluation of the association of positive patients and kidney rejection.

## METHODS

The 18 paediatric patients with kidney transplant who applied to Gazi University Hospital, Paediatric Nephrology Unit from 1 March 2014 to 1 August 2015 were included in the study. Viral nucleic acids were extracted from the samples using MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostics, Germany) in MagNA Pure Compact Instrument (Roche, Germany) device. Viral DNA amplified by using amplification mix Light Mix® Polyomavirus (BK/JC) Detection Kit (TIB Molbiol GmbH, Germany) that includes BKV, JCV primers and hybridization probes (Roche Diagnostics, Germany) in Light Cycler 2.0 (Roche Diagnostics, Germany) device.

## RESULTS

The rate of 27.8% (5/18) polyomavirus DNA positivity was detected in clinical samples of patients. Twenty two percent (4/18) of positive patients was detected as BKV positive, and 5.5% (1/18) was JCV positive. Kidney transplantations were done from alive donors and cadavers, 66.7% (12/18) and 33.3% (6/18) respectively. All of the polyomavirus DNA positive patients were transplanted kidney from alive donors. Quantitative BKV DNA amounts of 3 BKV positive patients were 103 copy/ml and of 1 BKV patient was 104 copy/ml. JCV DNA was detected by qualitative method. Kidney rejection has occurred at one of the polyomavirus positive patient who has JCV DNA. Urine cultures of polyomavirus positive patients were negative. Ciprofloxacin or sifodovir were used for the treatments of polyomavirus positive children.

## CONCLUSIONS

Early diagnosis and treatment possibility can be provided by real time PCR method to prevent rejection in transplant patients. Treatments of 5 positive patients were started immediately; however one patient has lost the transplanted kidney. The other 4 patients were treated successfully.

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# **PRE-TRANSPLANT CYTOMEGALOVIRUS (CMV) IGG ANTIBODY LEVELS COULD PREVENT SEVERE CMV INFECTIONS POST TRANSPLANT IN LIVER TRANSPLANT RECIPIENTS: EXPERIENCE FROM A TERTIARY CARE LIVER CENTRE.**

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## **BACKGROUND-AIM**

Humoral immune responses in Cytomegalovirus (CMV) infection are not studied well. Pre-transplant CMV IgG antibody levels (Pre-Tx IgG) could influence occurrence of post-transplant CMV infections. Therefore, correlation between pre-Tx IgG and post-Tx risk of acquiring CMV infection was investigated.

## **METHODS**

A total of 155 consecutive LTx recipients were included. Patients were not on anti-CMV prophylaxis. Nine cases died during the follow-up, and thus the final analysis included 146 patients. Pre-transplant donor (D) and recipient (R) IgG were estimated and post-transplant follow-up for CMV infection was done for one year.

## **RESULTS**

D+R+ serostatus was seen in 142 (97.3%) and D-R+ in 4 (2.7%). A total of 113 (77.4%) recipients had pre-Tx IgG of  $\geq 250$  AU/ml. Overall post-Tx CMV infections were seen in 54 (36.9%) recipients. In 32 (59.2%) patients CMV infection was seen during 1st month after Tx. Incidence of post-Tx CMV infection in recipients with pre-Tx IgG  $< 250$  AU/mL and  $\geq 250$  AU/mL were 42.4% and 34.5%, respectively ( $p = 0.99$ ). Median viral load was significantly higher in patients with pre-Tx IgG  $< 250$  AU/ml :  $4 \log_{10}$  (R: 2.8 -6.6  $\log_{10}$ ) copies/ml than those with  $\geq 250$  AU/ml :  $2.2 \log_{10}$  (R: 1.6-3.8  $\log_{10}$ ) copies/ml,  $p$  value=0.04. There was no difference in the time of occurrence of CMV infection in both the groups. Concurrent occurrence of rejection and CMV infection was seen in significantly more patients 18/54 (32.7%) than in patients without CMV infection (12/99, 12%,  $p = 0.002$ ).

## **CONCLUSIONS**

Higher pre-Tx CMV IgG levels might prevent severe CMV infections post-Tx. Recipients with low pre-Tx CMV titer might be benefitted by CMV prophylaxis or aggressive preemptive treatment.

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# **INVESTIGATION OF EBV INFECTION IN TRANSPLANT AND ONCOLOGY PATIENTS BY REAL TIME PCR**

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## **BACKGROUND-AIM**

Epstein-Barr virus is a herpesvirus that causes infectious mononucleosis, various lymphomas along with post transplant lymphoproliferative disease in immunosuppressive patients such as organ and tissue transplant recipients. The aim of our study is to investigate EBV DNA loads retrospectively in immunosuppressive patients who were transplant recipients or oncology patients.

## **METHODS**

Our study included blood and CSF samples of 341 patients, 185 female and 156 male, aged between 0-87, who applied to Gazi University Paediatric Gastroenterology, Haematology and other clinics between April 2014 and March 2017. EBV IgM, IgG antibodies and EA were investigated by ELISA (DIAPRO, Italy) in samples of patients that required serological analyses. DNAs were extracted by a commercial kit (High Pure Viral Nucleic Acid Kit, Roche, Germany). The isolated DNAs were amplified by Real Time PCR (LightMix Kit, Roche, Germany) in LightCycler 2.0 (Roche, Germany) and results were quantitatively evaluated.

## **RESULTS**

Totally 754 specimens (638 serum, 59 BOS, 4 BAL, 1 pericardial fluid, 1 pleural fluid, 3 eye fluid and 2 right and left lung fluid of the same patient) were sent to our lab. EBV DNA was found positive in 10.2% (77/754) of all samples tested. Among positive patients, 36.8% (14/38) were liver transplant patients, 5.2% (2/38) were renal failure patients, 5.2% (2/38) were bone marrow transplant patients, 2.6% (1/38) were acute myeloid leukaemia and 7.8% (3/38) were lymphoma patients. EBV DNA positive 23 (60.5%) patients' serologic results were available. Among them, 9 were VCA IgM, 7 were EA positive. VCA IgM and EA were both positive in 3 of the 9 patients and VCA IgM negative 2 patients were positive for EBNA IgM.

## **CONCLUSIONS**

In our study, EBV DNA was evaluated by Real Time PCR method since the vast majority of the individuals in our country have EBV seropositivity. In such patients, quantitative EBV DNA testing takes a great importance in terms of patient follow-up and prognosis. EBV infection is a high risk factor among immunosuppressive patients. Close clinical surveillance of EBV DNA in immunosuppressive patients is important for early diagnosis. Observing EBV DNA levels by real-time PCR assists evaluating the changes in clinical course.

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### GENETIC AND FUNCTIONAL PROPERTIES OF HEPATITIS C VIRUS VARIANTS THROUGH MOTHER TO CHILD TRANSMISSION

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#### BACKGROUND-AIM

In infected individuals, HCV circulates as a complex mixture of genetically different, but closely related viral variants. Envelope glycoproteins (E1E2) are the most variable regions and have a high immunogenicity. The rapid HCV evolution in a single host favors the emergence of mutants that can escape from specific immunity. In a transmission event (for example during the delivery), some viral variants are preferentially transmitted. The aim of our work was to identify molecular determinants of E1E2 associates with a greater capacity of transmission. We also intend to study the functional properties of transmitted and no transmitted variants, as for example sensibility to autologous neutralization.

#### METHODS

Studied sera samples were obtained from three women and their child infected by the HCV, who were participating in a clinical trial for the prevention of perinatal transmission of HIV in Thailand. Quasispecies were studied with single genome amplification to detect minor variants. Unique genomes were sequenced using Illumina technology. E1E2 sequences were analyzed on Biomina Galaxy platform allowing a de novo assembly with Trinity software.

#### RESULTS

Retroviral pseudotypes (HCVpp), bearing each transmitted and non-transmitted envelope glycoproteins were produced. For each one, the level of infectivity on HuH7 cells was measured, as well as the neutralizing activity of the autologous sera. For the first pair, the transmitted variants are resistant to autologous neutralization (DI80 <50). For the second pair, the non transmitted major variant is sensitive to autologous neutralization (DI80>800). The same experiments are currently in process for the third pair.

#### CONCLUSIONS

We hope that these results may be helpful to better understand early steps of HCV infection, which is of great interest for the development of immunoprophylaxis and vaccine strategies.

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### VARIABILITY IN RUBELLA SEROLOGICAL ASSAYS

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#### BACKGROUND-AIM

Rubella virus infection during the first trimester of pregnancy can result in congenital rubella syndrome. Although the incidence of rubella virus has decreased dramatically since vaccine introduction the impact on the individual remains high, as clinical decisions, such as termination of pregnancy, are based on serological diagnostic assays where a titre below 10IU/mL is considered at risk. Assays are, in many cases, standardised or traceable to the Anti-Rubella Immunoglobulin, Human WHO International Standard (IS) and report result in IU/mL.

#### METHODS

To assess the consistency in rubella diagnostic serological assays control reference materials and plasma packs from anonymised UK blood donors were tested across different EIA platforms and immunoblot for anti-rubella IgG. The reactivity of the same materials in functional assays, such as SRH was also determined and all results reported in IU/mL.

#### RESULTS

NIBSC archive data show the anti-rubella titre in blood donors has decreased from 115 to 62 IU/mL from 2007 to 2016. For selected samples collected in 2016 it was seen that the concentration of anti-rubella antibodies reported was dependent upon the assay used; selected samples were reported negative by EIA when they have SRH reactivity and equally samples with no SRH reactivity having high IU/mL anti-rubella IgG reported by EIA.

#### CONCLUSIONS

In conclusion, assays for rubella diagnosis require further standardisation. The relative clinical value of anti-rubella antibody detected in EIA and functional assays needs to be established. The Anti-Rubella International Standard was introduced before the common use of EIAs and an IS is required for continued efforts to harmonise assays. Further secondary standards are required to facilitate standardisation of rubella assays that are representative of the types of serological tests used on today's vaccinated populations and inform whether individuals are protected against rubella infection.

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### THE FIRST CASE OF ZIKA VIRUS RELATED MICROCEPHALY IN THE NETHERLANDS

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#### BACKGROUND-AIM

Recently, following an outbreak in Brazil and subsequently spread throughout the Americas, Zika virus (ZIKV) became an important infectious disease linked to congenital neurologic birth defects, including microcephaly.

#### METHODS

Case description and diagnostics of ZIKV related microcephaly.

#### RESULTS

A 30 year old primigravid was referred to our tertiary hospital for evaluation at 30 weeks gestational age because of suspected microcephaly. Repeated neurosonography showed a head circumference of 217mm ( $p < 3$ ), abdominal circumference of 245mm ( $p3-p10$ ) and a femur length of 55mm ( $p10$ ). Transvaginal evaluation of the fetal brain showed signs of severe bilateral cerebral damage including subcortical bandlike calcification, enlarged subarachnoidal space and lack of cortical folding, especially in the frontal lobes. Fetal MRI confirmed these findings. The routine 20 weeks scan was re-evaluated and showed a head circumference on the third percentile with a normal intracranial anatomy. Patient reported that she and her partner visited Mexico at a gestational age of 10 weeks. Her partner experienced a systemic rash, fatigue and signs of conjunctivitis during the holiday and the patient developed a systemic rash 2 weeks later, after returning home at week 12 of gestation. Maternal serology, performed at 30 weeks gestation, was positive for IgG and negative for IgM antibodies against ZIKV. In retrospect, seroconversion could be observed between week 10 and 32 of gestation with a high IgG neutralizing antibody titer of 1:1290 against ZIKV in week 32. In addition, amniotic fluid taken in week 32 was tested positive for ZIKV RNA on semiquantitative RT-PCR, compatible with a ZIKV infection of the fetus.

After extensive counseling the parents opted for a third trimester termination of pregnancy. Postnatal MRI confirmed the severe brain injury, subcortical bandlike calcification, enlarged subarachnoidal space and lack of cortical folding. Serum taken before termination of the pregnancy and postmortem fetal brain tissue were also tested positive for ZIKV RNA, confirming the diagnosis.

#### CONCLUSIONS

As of November 2016, ZIKV infection during pregnancy is a notifiable disease in the Netherlands with 14 registries so far. Here, we present the first complicated case of ZIKV related microcephaly in the Netherlands.

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### ANTIRETROVIRALS QUANTIFICATION DURING PREGNANCY: FROM THE ANALYTICAL DEVELOPMENT TO CLINICAL APPLICATION

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#### BACKGROUND-AIM

The highly active antiretroviral therapy (HAART) is the main resource to avoid the mother-to-child transmission of HIV. The physiological changes during pregnancy are factors to predispose to significant changes in plasmatic concentration. In this context, the quantification of HAART during pregnancy and the association with the therapeutic response is of critical importance.

#### METHODS

The detection of antiretroviral drugs was performed using tandem mass spectrometry applying multiple reaction monitoring (MRM). We used positive electrospray mode: LMV (230.18/112.08), ZDV (268.22/127.10), LPV (629.55/447.35), and RTV (721.50/296.20). Chromatographic separation was carried out with a BEH C18 (2.1mmx50mm, 1.7mm) column, with acetonitrile and 0.1% formic acid gradient. The injection volume was 3µL and run-time was 3.0 min. The process for plasma samples consisted of protein precipitation using 0.1% formic acid in cold acetonitrile. For the breast milk samples, first we performed a lipids extraction using hexane, then protein precipitation. Both methods were validated in accordance with the Mexican standards and FDA criteria. Finally, ten plasma samples from eight HIV-positive women, in the third trimester of pregnancy and four colostrum samples were assayed.

#### RESULTS

Methods validation were conducted over a range (ng/mL) between 50-3000, 75-4500, 250-15,000 and 25-500 for LMV, ZDV, LPV and RTV for plasma samples. For colostrum samples the range was between 50-2,500, 12.5-750, 100-6,000 and 5-300 ng/mL for LMV, ZDV, LPV and RTV. In plasma samples, we did not detect antiretroviral drugs in three samples (3/10). A patient had undetectable levels of HAART in two samples obtained in different time of pregnancy, suggesting non-treatment adherence. The colostrum samples revealed higher quantities than reported in literature.

#### CONCLUSIONS

These methods are selective, accurate and exact for simultaneous quantification of four antiretroviral drugs LMV, ZDV, RTV, and LPV in plasma and colostrum samples. These methodologies can be used in therapeutic antiretroviral drugs monitoring, characterizing the drugs excretion trough breast milk and the approach to breastfeeding the children from HIV-positive women, and in the measure of treatment adherence in pregnant women.

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### SERO-PREVALENCE OF HUMAN PARVOVIRUS B19 AMONG REPRODUCTIVE AGED WOMEN IN FIVE DIFFERENT CHINESE PROVINCES

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#### BACKGROUND-AIM

Human parvovirus B19 (B19V) is a small single-stranded DNA virus, most commonly spread by respiratory secretions; however, infection from blood products as well as prenatal vertical transmission can also occur. Infection with B19V during pregnancy is known to be associated with fetal conditions such as hydrops fetalis and intrauterine fetal death. The aim of this study is to assess the sero-prevalence of B19V among the pregnant women in different Chinese provinces, the correlation with demographic factors and to compare the results to those of other studies already published.

#### METHODS

From July to December 2016, 1401 women aged from 18 to 42 years old from five cities in China were included in the study and divided in the following categories: pre-pregnancy, early pregnancy, mid-pregnancy and late pregnancy. Specific IgG and IgM antibodies were measured using LIAISON® Biotrin Parvovirus B19 IgG and IgM chemiluminescence Immunoassay (DiaSorin, Italy).

#### RESULTS

The overall prevalence of IgG and IgM in the studied population was 15.2% and 0.4% respectively. The IgG positive rate of women in Beijing, Chongqing, Shanghai, Shenzhen and Shenyang was 24.0%, 18.9%, 17.9%, 14.2% and 10.2%, respectively. Distribution of IgG positive women in different pregnant stages was not statistically different ( $P > 0.05$ ). However, the positive rates of parvovirus B19 IgG in the early pregnancy in the five cities were 26.23%, 18.75%, 15.97%, 7.84% and 10.11% correspondingly, showing significant difference. And the IgG positive rate in the late pregnant period in Shanghai was significantly higher than that in Shenyang ( $P < 0.01$ ). The positive IgG and IgM rate was lower than those previously reported in other Chinese and worldwide studies [Na et al, 2014; ACOG, 2015; Li et al, 2013; Neu et al, 2015].

#### CONCLUSIONS

The results obtained in our study confirm the significant percentage of child-bearing aged women in the different Chinese provinces, who are at risk of primary infection with B19V that could adversely affect their pregnancy. Besides methods used in different studies, the lower IgM positive rate reported may be attributed to the period of year in which the samples were collected.

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### EVALUATION OF RECOMLINE CMV IGG [AVIDITY] ASSAY FOR DETERMINATION OF CMV IGG AVIDITY DURING PREGNANCY

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#### BACKGROUND-AIM

Cytomegalovirus (CMV) primary infection during pregnancy is at particular risk of transmission of the virus to the fetus, and subsequent congenital disease. It is therefore important to confirm or exclude recent CMV primary infection when CMV-IgG and CMV-IgM are positive, in order to offer proper management of the pregnancy. Measuring CMV-IgG avidity index is mainly used for this purpose, and our objective was to evaluate performances of an original assay (recomLine CMV IgG [Avidity] developed by MIKROGEN (Neuried, Germany).

#### METHODS

Avidity determination with recomLine CMV IgG [Avidity] assay is performed by a comparative evaluation of two CMV-IgG strips tested in parallel with the same sample (only one of the two strips is treated with the avidity reagent), as recommended by the manufacturer.

Performances of the kit were evaluated with 160 serum of pregnant women classified in three groups depending on the time of collection following CMV infection: group 1 (N=50) collected more than 12 weeks after onset of infection; group 2 (N=50) collected less than 12 weeks after onset of infection; group 3 (N=60) collected at different stages of infection (including at least 3 samples per patient). Analyzes were done in comparison with our routine assays: LIAISON®CMV IgG Avidity II, DiaSorin and VIDAD CMV IgG Avidity II, bioMérieux.

#### RESULTS

For group 1, when high avidity results were expected, a concordance of 87% was observed between recomLine CMV IgG [Avidity] assay and our routine assays. In few cases, the recomLine CMV IgG [Avidity] assay was inconclusive (6.5%) or discordant (6.5%). For these samples complementary investigations were performed. For group 2 and 3, almost all samples were correctly classified (93%) as primary infections. Interestingly, no sample was wrongly classified as a past CMV infection with the recomLine CMV IgG [Avidity] assay. However, in few cases of samples collected very early after seroconversion, no CMV-IgG were detected with the recomLine CMV IgG (7%).

#### CONCLUSIONS

In our experience, recomLine CMV IgG [Avidity] assay reliably classifies recent and past CMV infections. In order to assess if it could help to clarify unclear situations, when CMV-IgG avidity performed with other assays is moderate, supplementary samples will be tested.

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# CHARACTERIZATION OF MYCOPLASMA, UREAPLASMA AND HUMAN PAPILLOMAVIRUS INFECTIONS IN ITALIAN FERTILE AND INFERTILE WOMEN

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## BACKGROUND-AIM

Mycoplasma and Ureaplasma are considered opportunistic pathogens of human urogenital tract and their implication in infertility was still a controversial matter of discussion. Co-infection with other sexually transmitted pathogens, as such as Human Papillomavirus, has been observed due to the increase susceptibility of vaginal mucosa by the local chronic inflammation. Recent reports suggested a role for Ureaplasma/ Mycoplasma in the early phase of HPV pathogenesis. The aim of this study was to characterize Mycoplasma/Ureaplasma infections in sexually active Italian women according to demographic, individual risk factors, sexually transmitted infections (STI) and infertility conditions.

## METHODS

A selected group of 521 women were recruited from Assisted Reproductive Technology centre, with a diagnosis of infertility. In addition, 125 women with proven fertility history giving birth, included 65 pregnant women attended from the Gynecology Service and 60 symptomatic women attended to the Sexually Transmitted Disease center, were enrolled. A qualitative real time PCR was performed to assess the genomic sequences of Mycoplasma/Ureaplasma species and of other STI.

## RESULTS

Analysis of prevalence considering fertility demonstrated that Mycoplasma/Ureaplasma infection was more present in Fertile women (59,2%) respect to in Infertile women (31%). U. parvum represented the specie most frequently detected, with a highest bacterial load in Fertile STD women. Among STI pathogens, a high prevalence of HR-HPV was found in STI symptomatic (46,6%) and asymptomatic pregnant women (30%), while no sequence of this virus was detected in Infertile women. Data analysis showed that U. parvum was preferentially associated with the other STI infections, particularly with HR-HPV, both in STI symptomatic (31%) ( $p<0,01$ ) than in asymptomatic pregnant women (13%) ( $p<0,05$ ).

## CONCLUSIONS

Mycoplasma/Ureaplasma infections were not associated with women infertility, while a positive correlation was found only with STI. To note, the strong association with HR-HPV focused the attention on the importance to detect this microorganism in sexually active women, since its presence could alert a asymptomatic HR-HPV infection.

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# THE IMPACT OF PRIMARY VS SECONDARY HCMV INFECTION IN PREGNANCY FOR THE OUTCOME IN THE NEWBORN

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## BACKGROUND-AIM

Mother-child infection of HCMV is the most leading course of birth defects and developmental disabilities worldwide. The highest risk for adverse outcome in the newborn results from primary infection during the first term of pregnancy. In most of the countries worldwide as in Africa and Asia there is a high seroprevalence of HCMV antibodies in young adults. Symptomatic HCMV congenital infection born by mothers with preexisting antibodies are published from Alabama and Sao Paulo. The rate of bad outcome in these studies in the newborns was about the same (10%) like in primary infection. Secondary HCMV infection is highly dependent in lifestyle such as close contact to children < 3 years and the number of sexual partners during pregnancy. These HCMV exposures increase the risk to acquire a secondary infection with another HCMV gp-type.

## METHODS

In a recent study of HCMV seropositive mothers in China, done in two hospitals of Shanghai, we found that 0.9% of investigated DBS of the newborns were positive by PCR at the time of birth. Follow ups were done for clinical symptoms and laboratory findings of the HCMV infected children. In ten of the 17 PCR positives, the clinical investigation could be done.

## RESULTS

None of the positive newborns had clinical symptoms at birth for HCMV infection. All newborns were screened at birth between 2011 to 2012 in Obstetrics and Gynaecology Hospital of Fudan University and the Community Health Center of Pujiang Town.

## CONCLUSIONS

These data from Shanghai are in agreement with recently published work from the Shandong province (Wang et al. Medicine, 2017, 96 (5): 1-6) were 0.7% of HCMV congenital infection were found and not a single newborns did demonstrate symptomatic HCMV infection. Further studies on the the prevalence of congenital HCMV ifnection in high prevalence countries with follow-ups are necessary to define in more detail the burden of HCMV disabilities in newborns. Based on the data from Alabama and Sao Paulo, obtained from women with high seroprevalence, a total misscalculating comes up for the burden of congenital HCMV disease worldwide.

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### ENGAGEMENT OF PARVOVIRUS B19 IN THE DEVELOPMENT OF ANEMIC SYNDROME IN WOMEN WITH ABNORMAL PREGNANCY

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#### BACKGROUND-AIM

Viral infections during pregnancy are a leading cause of severe complications and mortality of mother and fetus. This study aims to analyze the participation of parvovirus B19 as a etiologic agent in the development of anemic syndrome, support a proper differential diagnosis and improve prognostic and treatment of women with abnormal pregnancies and newborns in Bulgaria.

#### METHODS

For the period 03.2015- 11.2016 were examined 67 serum samples of pregnant women with abnormal pregnancy, treated in SBALAG " Maichin Dom " Sofia. Patients were divided into four groups: pregnant women with anemia (n = 22), non-immune hydrops fetalis (n = 8), fetal ascites (n = 6) and women suffered a miscarriage (n = 31). The study included three newborns (n = 3), tested on the occasion of a possible maternal-fetal infection. Serological (indirect ELISA test) and molecular (B19V-PCR test) methods were used. CLIA, AAS, NEPH methods were included to determined indicators of iron homeostasis among patients with anemia.

#### RESULTS

A total of 6/67 (8.95%) patients were confirmed with primary reactive B19V-IgM antibodies. Among evaluated pregnant women with anemia, non-immune hydrops fetalis, fetal ascites and miscarriage the rate of detected B19V-IgM antibodies was as follows: 18,2% (4/22), 12.5% (1/8) and 16.7% (1/6 ) and 0% (0/31). Protective B19-IgG antibodies were found in 25/67 (37.3%) samples. In all patients with positive B19V-IgM antibodies viral DNA was found by PCR analysis. All three tested newborns were positive for B19V -IgG antibodies (mother), while one was demonstrated B19V-IgM antibodies, and presented viral DNA. Anemia was determined as iron-deficient according to the low serum levels of hepsidin  $2.54 \pm 0.4 \mu\text{g} / \text{l}$  compared to  $20.9 \pm 2.8 \mu\text{g} / \text{l}$  in the control group of pregnant women without anemia.

#### CONCLUSIONS

Molecular - diagnostic approach to analyzing B19V showed the highest rate of engagement of that virus in the development of abnormal pregnancy in women with anemic syndrome. Determination of serum hepsidin in pregnant women with parvovirus B19 infection would help to clarified the etiological impact of the anemic syndrome and prevents improper supplementation with iron during pregnancy.

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### INVESTIGATION OF HUMAN PARVOVIRUS B19 DNA POSITIVITIES BY REAL-TIME PCR IN PREGNANT WOMAN

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#### BACKGROUND-AIM

The purpose of this study is investigation of Parvovirus B19 DNA by real time PCR in samples sent from Gyneacology Clinics to our laboratory and evaluation of association of clinical state and viral load.

#### METHODS

The samples sent from Gazi University Hospital, Gyneacology Clinic to Molecular Microbiology Lab., between July 2014-December 2016 were included in the study. Parvovirus B19 IgM and IgG antibodies were investigated by ELISA (NovaTec, Germany) in clinical samples of patients that required of serological analyses. Serological profiles of patients were determined and viral nucleic acids were extracted from the samples using MagNA Pure Compact Nucleic Acid Isolation Kit, in MagNA Pure Compact Instrument (Roche Diagnostics, Germany) device. Viral DNA amplified by Real Time PCR quantitatively, using Light Mix® Parvovirus B19 Detection Kit (TIB Molbiol GmbH, Germany) that includes B19 primers and hybridization probes in Light Cycler 2.0(Roche Diagnostics, Germany) device.

#### RESULTS

Five blood and 5 amnion fluid samples from 9 patients were sent for B19 DNA detection. Among those 1 patient had B19 DNA positivity in her both blood and amnion fluid samples, however B19 IgM was negative and B19 IgG was positive. The other 8 patients were negative for B19 DNA. Totally 5 patients' serological test results were available, B19 IgM result was 'grey zone' for only 1 patient and patients had positive B19 IgG results. B19 DNA positive patient was 22 weeks pregnant and 106 copy/ml and 103 copy/ml B19 DNA was detected in her amnion fluid and blood, respectively. Her amniocentesis result was normal and fetus was healthy after delivery. Among the 8 patients, pregnancy was terminated in 3 patients with fetal anomaly and 1 patient had spontaneous abortion at 8th week. Two patients diagnosed with hydrops fetalis at their 22nd and 27th weeks, however their B19 DNA results were negative. Another patient diagnosed with fetal hydrothorax and the other 2 patients had fetal distress and fetal problems as prediagnosis, respectively.

#### CONCLUSIONS

B19 virus is transmitted through vertical transmission to the fetus and creates some complications to fetus. Fetal infection is unlikely to occur if the mother is immune at the time of exposure, because of passively acquired maternal immunity.

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### CONGENITAL CYTOMEGALOVIRUS INFECTION: PROGNOSTIC VALUE OF REAL-TIME PCR PERFORMED ON DRIED BLOOD SPOTS

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#### BACKGROUND-AIM

Cytomegalovirus (CMV) is the leading cause of congenital infection in humans and a major cause of sensorineural hearing loss and neurologic impairment in children. Several studies have been performed to identify predictive factors of short- and long-term outcome in congenitally CMV infected infants. A high viral load, as measured by real-time quantitative polymerase chain reaction (qPCR), either in fetal amniotic fluid or in neonatal blood and urine, has been reported to correlate with future damage in the fetus and in the child.

The aim of this study was to define the prognostic value of qPCR performed on dried blood spots (DBS) collected on filter paper (Guthrie-card) in congenitally CMV infected infants.

#### METHODS

CMV infected infants were followed-up for at least 3 years: virologic tests, clinical, psychometric, ophthalmologic and audiologic evaluations were performed at birth, at 3-6-12-18 months of age, and annually thereafter. Guthrie-cards performed in the first days of life for neonatal screening of genetic and metabolic disorders were subsequently obtained from the regional screening laboratory for all the study subjects and tested for CMV by qualitative and quantitative PCR (nested and qPCR).

#### RESULTS

A cohort of 65 CMV infected infants was studied (mean gestational age 37.8 weeks, mean born weight 2710 g). 18/65 (27.7%) were symptomatic at birth; 8 of them (44.4%) developed sequelae. 5/47 (10.6%) asymptomatic infants developed sequelae. Nested PCR was positive in 93.8% (61/65) and qPCR in 95.4% (62/65) of all infants studied. The viral load determined by qPCR was  $\geq 103$  copies/mL in 68.4% of infants with symptoms at birth vs. 33.8% of those without symptoms ( $p < 0.005$ ) and in 62.5% of infants who developed sequelae vs. 34.9% of those who did not ( $p < 0.05$ ) (chi square test).

#### CONCLUSIONS

DBS test using real-time qPCR seems to be a useful method to identify infants at high risk of sequelae. The ability to identify children who are at increased risk for neurologic impairment should provide a basis for more timely and appropriate counselling for parents, permit careful monitoring and aid in formulation of interventional strategies.

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### A SALIVA PCR BASED SCREENING IN NEWBORNS FOR CONGENITAL CMV INFECTION IN IZMIR, TURKEY.

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#### BACKGROUND-AIM

Human cytomegalovirus (HCMV) infection is common and IgG seroprevalence is about 90 % in Turkey. Congenital cytomegalovirus (CCMV) infection occurs in 0.2- 2.5 % of births causing long-term sensorineural hearing loss (SNHL) and neurological impairment in a significant proportion of infected infants. Congenital CMV infection occurs in 0.2 to 2.2% of live-borne neonates. A significant proportion of infected infants do not present with symptoms and might only be detected by routine screening methods. Accurate and timely diagnosis of CCMV is essential to allow prompt recognition of sequelae and provide the opportunity for treatment. The aim of our study was to screen live-born neonates with a saliva real-time polymerase-chain-reaction (RT-PCR) for the detection of CMV.

#### METHODS

Saliva samples of the 689 newborn was collected by the pediatrician in 20 minutes after birth using an air dried dacron swab (Cultiplast, Italy). After elution and incubating at 95°C, internal control was added and automated real-time PCR (RT-PCR) was performed in Abbott m2000 sp (Abbott Molecular, USA). If positive on saliva, CMV infection was confirmed by performing the same RT-PCR on a urine or plasma sample of the newborn within three weeks.

#### RESULTS

In our hospital 689 newborns were screened for CMV DNA in saliva. Eight of 689 (1.16 %) of the newborns were CMV DNA positive. Two ( 0.29 %) were considered true cases of congenital CMV, six newborns were interpreted to be false positive based on urinary and or plasma confirmation. Cycles of threshold (ct) values of the RT-PCR were 14.8 and 29.3 for true positive and between 29.6-30.3 for false positive CMV DNA results. One of the congenital CMV newborns (the one with a higher viral load) failed sensorineural test and was symptomatic.

#### CONCLUSIONS

In our study dry saliva based screening for CMV DNA immediate after births revealed 1.16 % CMV DNA positivity and congenital CMV rate was 0.29 %.

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### VIRUS AND ANTIBODY DYNAMICS IN TRAVELERS WITH ACUTE ZIKA VIRUS INFECTION

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#### BACKGROUND-AIM

To improve our understanding of the natural history of ZIKV infection in humans, we described the dynamics of ZIKV RNA shedding in different bodily fluids and antibody responses in patients with acute infection.

#### METHODS

We enrolled 30 adults with acute ZIKV infection, including 29 with travel-associated infection and one case of sexual transmission; 3 participants were asymptomatic, 2 were pregnant, and 5 had previous dengue. Follow-up evaluation included weekly collection of blood, urine, saliva and semen samples for ZIKV RNA and antibody testing. Follow-up was continued until ZIKV RNA tests become negative and antibodies were detectable in serum.

#### RESULTS

ZIKV RNA was detected in plasma of 57% of participants with relatively low viral load. The estimated median time to ZIKV RNA clearance from plasma was 11.5 days, IQR 6-24. The two pregnant women had persistent ZIKV RNA detectable in blood until delivery of apparently healthy infants. Shedding of ZIKV RNA in urine and saliva occurred in 93.1% and 69.2% of participants, respectively, and reached peaks with high loads about one week after onset. Median times to ZIKV RNA clearance in urine and saliva were 24 days, IQR 17-34, and 14 days, IQR 8-31, respectively. ZIKV RNA was detected in semen of 5/10 tested men, with very high viral load in some cases. Median time to ZIKV RNA clearance from semen was 25 days, IQR 14-29. One patient had detectable ZIKV RNA in semen for 370 days after symptom onset. Based on the results of ZIKV NS1 IgM and IgG ELISA, 40% of participants were seronegative at the time of first laboratory evaluation and further 17% of cases (those with previous DENV infection) had already ZIKV IgG but remained IgM-negative during follow-up. In flavivirus naïve patients, the median times to detection of ZIKV-specific IgM and IgG antibodies were estimated as 8 days, IQR 5-15, and 17 days, IQR, 12-26, respectively.

#### CONCLUSIONS

Prolonged viremia and ZIKV RNA shedding in urine, saliva, and semen occur frequently in patients with acute ZIKV infection. At the time of diagnosis, about half of patients are ZIKV IgM-negative. ZIKV NS1 IgM antibodies remain undetectable in patients with previous dengue. Estimates of the times to viral clearance and seroconversion are useful to optimize diagnostic algorithms.

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### A MODEL FOR GLOBAL DETECTION OF EV-D68 OUTBREAK THROUGH A CLOUD BASED EPIDEMIOLOGY NETWORK

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#### BACKGROUND-AIM

Real time surveillance of pathogens can facilitate management of outbreaks and enrich understanding of disease dynamics. The BioFire FilmArray® Respiratory Panel (RP) can detect 20 respiratory pathogens including rhinovirus/enterovirus (HRV/EV). FilmArray® Trend is an epidemiology tool that automatically receives de-identified test results from the FilmArray® Systems. In 2014, enterovirus (EV) D68 was associated with an outbreak of severe respiratory illness in children worldwide resulting in some cases with Acute Flaccid Myelitis. Although HRV/EV is not subtyped by the FilmArray RP, we have developed a model for EV-D68 identification using the (masked) PCR data exported to the database from 2014-2016.

#### METHODS

52,000 HRV/EV positive test results were found in the FilmArray Trend dataset. We examined patterns within the real time PCR data from the six HRV/EV assays and developed an algorithm for detecting EV-D68: the Pathogen Extended Resolution (PER) test. One European site in Groningen, Netherlands and four US sites contributed FilmArray data files from samples that were independently characterized by an EV-D68 specific PCR test or by sequencing. This data was used for algorithm tuning and evaluation.

#### RESULTS

The percent positivity for the PER test, trained to identify EV-D68, demonstrated an overall sensitivity of 91% and specificity of 91% when evaluated against 713 US clinical samples. This algorithm demonstrated 96% sensitivity and 86% specificity on 59 samples from the Netherlands collected in 2014 and 2016. When plotted over time, the percentages of predicted EV-D68 per regional RP tests were generally low with occasional regional outbreaks predicted in 2014 and 2016.

#### CONCLUSIONS

We have demonstrated that the PCR amplification data available to BioFire through the FilmArray Trend database can be used to identify signatures associated with EV-D68 in both the US and EU over a 3-year period. Development of the PER Test to identify patterns masked within the FilmArray® Software, has the potential to provide alerts to network participants of anomalous patterns in pathogen trends. Further validation, development and refinement of these alert algorithms and how to use them must be explored before broad implementation.

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### THE CHALLENGES AND FINDINGS FROM THE FIRST YEAR OF ENHANCED SURVEILLANCE FOR NON-POLIO ENTEROVIRUSES IN WALES

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#### BACKGROUND-AIM

There is increased interest in developing a European network of collaborating centres for the enhanced surveillance of non-polio enteroviruses. Success involves developing a consensus regarding case definitions, sample types, laboratory methods, data analysis and reporting. As the non-polio enteroviruses are not currently part of a global surveillance strategy, funding and capacity is also an issue especially in resource poor countries or organisations. Therefore, moving forwards there needs to be agreement on what the surveillance should target and how this might be supported in all regions to prevent data black-spots.

We describe the challenges and findings from the first year of implementation of enhanced enterovirus surveillance for Wales, that was set-up in response to this increased awareness.

#### METHODS

Public Health Wales systematically collects clinical details and demographics of all enterovirus positive cases detected in Wales (population: 3 million). From July 2016, all respiratory samples were tested by a specific enterovirus assay and then typed to EV-D68 by real-time PCR. VP1 partial sequencing was performed on all positive samples for further characterisation. Additionally, a separate project stream was undertaken to perform whole genome sequencing specifically targeting the respiratory samples. All of the data was collated and analysed to look for trends and to provide a baseline of enterovirus circulation in Wales.

#### RESULTS

By June 2017, information on 390 enterovirus cases from across Wales had been collected. Of those detected in CSF and lesion swabs, 95% were successfully typed by VP1 sequencing. A significant proportion of respiratory samples could not be typed by Sanger sequencing, so this was halted in favour of WGS. By testing all respiratory samples for enterovirus and EV-D68 two epidemic waves were noted in Wales. Cases of AFP associated with EV-A71 and a countrywide epidemic of CV-A6 was also observed. The echoviruses dominated in Wales during 2016/17 associated with meningitis and sepsis in neonates.

#### CONCLUSIONS

Implementing enhanced surveillance of non-polio enteroviruses is possible and allows for a wider understanding of the clinical spectrum of disease. However, such a scheme needs to be appropriately resourced to allow for a sustained service moving forwards.

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### CIRCULATION OF USUTU VIRUS IN HEALTHY FORESTRY WORKERS AND BLOOD DONORS IN LOMBARDY REGION, NORTHERN ITALY

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#### BACKGROUND-AIM

In 2009 in Northern Italy, the first human Usutu virus (USUV) neurological infections were reported in two immunocompromised patients. Seroprevalence data for USUV in Europe show circulation at low prevalence in human populations. Two consecutive studies in Northern Italy indicate a possible increase in the presence of the virus, from 0% to 0.23% in blood donors. The aim of this study was to investigate possible USUV infection in a group of guards of the "Ticino Valley Park" (Northern Italy) in comparison with a group of healthy blood donors living in the same area.

#### METHODS

In the present study, 33 park guards were monitored every six months from October 2014 till December 2015 for the detection of USUV, WNV and TBEV specific antibodies. This group was compared with 200 blood donors living in the same areas. Serum samples collected from each patient were tested by endpoint titration for USUV, WNV and TBEV IgM and IgG with an in-house indirect immunofluorescence assay and with a micro-neutralization assay (NT).

#### RESULTS

The USUV seroprevalence found in the park guards was 18.1% (6/33). No IgM antibodies were detected in any of the samples tested. Three workers were immune at the time of all serum samplings, while the other three exhibited seroconversion for IgG and NT antibodies during the study, without symptoms. IgG and NT antibodies against WNV were detected only in two of the six USUV positive cases of the workers group, with very low titres. Antibodies against TBE were negative in all the serum samples of forestry workers. The 200 samples from blood donors resulted negative for TBEV and WNV antibodies, while two were positive for USUV IgG by IFA and NT and IgM negative. The seroprevalence among this group was 1% (2/200).

#### CONCLUSIONS

The high prevalence (18.1%) of USUV antibodies in a high-risk group in the "Ticino Valley Park" emphasizes the need for monitoring the co-circulation for both USUV and WNV in this geographical area. In parallel, the prevalence found among blood donors (1%) in the same area is also higher than expected. The results of this study corroborate the hypothesis that USUV can cause clinically asymptomatic infection among humans and pose a possible problem for blood transfusion monitoring in areas where the virus is active.

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### A FULLY AUTOMATED IMMUNOASSAY FOR THE DETECTION OF ZIKA VIRUS IGM

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#### BACKGROUND-AIM

Zika virus is a mosquito-borne flavivirus. It has gained attention in the last few years due to the association between Zika virus infection during pregnancy and microcephaly. Virus-specific IgM antibodies are typically present after the first week of illness and may be detectable for up to 12 weeks. The detection of IgM antibodies to Zika virus provides an essential tool for diagnosis and follow-up care of an acute or recent infection. The objective of this study is the development and validation of a rapid, reliable and accurate automated immunoassay for the detection of Zika virus IgM antibodies in human sera.

#### METHODS

The LIAISON® XL Zika Capture IgM assay is an in vitro diagnostic chemiluminescent immunoassay intended for the qualitative detection of Zika virus IgM antibodies in human serum. The assay uses an antibody capture format with anti-immunoglobulin antibodies coated onto magnetic particles. Detection is by a recombinant Zika virus non-structural protein 1 (NS1), labeled with a isoluminol derivative. The assay is performed on the fully automated LIAISON® XL analyzer.

#### RESULTS

Clinical sensitivity of the LIAISON® XL Zika Capture IgM assay was evaluated using serially collected samples from 58 symptomatic subjects initially PCR positive for Zika virus. All subjects were detected by the assay as Zika IgM positive by the first sample collected following 8 days post-symptom onset. Zika IgM could be detected as early as 4 days and as late as 83 days post-symptom onset. Clinical specificity was evaluated using 218 apparently healthy donors and 32 pregnant donors collected in the United States and presumed negative for Zika virus. The assay was negative in 249 of the 250 samples, for a clinical specificity of 99.6%. Precision of the assay was evaluated with intra and total imprecision results of <9% and <14% respectively. The assay was also tested for cross reactivity to other related flaviviruses using samples that were positive for dengue virus or West Nile Virus IgM antibodies, or from subjects who had been vaccinated for yellow fever and no cross reactivity was detected.

#### CONCLUSIONS

DiaSorin's new LIAISON® XL Zika Capture IgM immunoassay offers a fully automated and precise alternative to the currently available manual ELISA assays for detection of Zika virus IgM antibodies

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### EVIDENCE OF INCREASING DIVERSIFICATION OF ZIKA VIRUS STRAINS ISOLATED IN THE AMERICAN REGION

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#### BACKGROUND-AIM

Zika virus (ZIKV) is a member of the family Flaviviridae. ZIKV triggered an epidemic of significant proportions in Brazil in 2015 and since its spread across the American region. Detailed phylogenetic studies are extremely important to understand the emergence, spread and evolution of ZIKV populations.

#### METHODS

A Bayesian coalescent Markov Chain Monte Carlo analysis of complete genome sequences of ZIKV strains recently isolated in the American region was performed.

#### RESULTS

The results of these studies revealed an increasing diversification of ZIKA virus strains in different genetic lineages and co-circulation of distinct genetic lineages in several countries in the region. The time of the most recent common ancestor (tMRCA) was established to be around February 15th, 2014 for ZIKV strains circulating in the American region. A mean rate of evolution of  $1.89 \times 10^{-3}$  substitutions/site/year was obtained for ZIKV strains included in this study. A Bayesian skyline plot indicate a sharp increase in population size from February, 2014 to March, 2015 and a decline during 2016.

#### CONCLUSIONS

Taking all these results together, high evolutionary rates and fast population growth characterize the population dynamics of ZIKV strains that emerged in the American region. Strains isolated from microcephaly cases do not share amino acid substitutions, suggesting that other factors besides viral genetic differences may play a role for the proposed pathogenesis caused by ZIKV infection.

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**GENOME-WIDE ANALYSIS OF CODON USAGE BIAS IN ZIKA VIRUS**J. Cristina<sup>1</sup>, A. Fajardo<sup>1</sup>, M. Soñora<sup>1</sup>, G. Moratorio<sup>1</sup>, H. Musto<sup>1</sup><sup>1</sup>Facultad de Ciencias, Universidad de la Republica, Montevideo, URUGUAY**BACKGROUND-AIM**

Zika virus (ZIKV) is a member of the family Flaviviridae. The relation of codon usage among viruses and their hosts is expected to affect viral survival, fitness, evasion from host's immune system and evolution.

**METHODS**

In this study, a comprehensive analysis of codon usage and composition of ZIKV was performed.

**RESULTS**

The overall codon usage among different ZIKV strains is similar and slightly biased. Different codon preferences in ZIKV genes in relation to codon usage of human, *Aedes aegypti* and *Aedes albopictus* genes were found. Most of the highly frequent codons are A-ending codons. G + C compositional constraint as well as dinucleotide composition also influence the codon usage of ZIKV.

**CONCLUSIONS**

The results of these studies strongly suggests that mutational bias is a main force shaping codon usage in this virus. Geographical and genetic variability may also play a role in shaping the molecular evolution and codon usage in ZIKV. No significant differences were found in codon usage among strains isolated from microcephaly cases.

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**DETECTION OF ZIKA VIRUS INFECTIONS IN TRAVELLERS RETURNING TO IRELAND IN 2016**G. Tuite<sup>1</sup>, M. Duffy<sup>1</sup>, G. Franzoni<sup>1</sup>, B. Keogan<sup>1</sup>, C. De Gascun<sup>1</sup>, J. Connell<sup>1</sup><sup>1</sup>National Virus Reference Laboratory (NVRL), University College Dublin, Belfield, Dublin 4, Ireland.**BACKGROUND-AIM**

There is an increasing association between Zika virus (ZV), spread by the *Aedes* mosquitoes, and congenital brain abnormalities including microcephaly, and Guillain-Barre syndrome. Autochthonous transmission within Europe has not been reported; however imported cases of ZV have been observed.

Aim: To detect ZV infection in travellers returning from endemic areas, to Ireland in 2016.

**METHODS**

Samples (urine, serum, plasma and semen) depending on the time post infection, and potential exposure, were tested for ZV RNA (Altona Realstar® ZIKA RT-PCR kit) and/or ZV antibodies (Euroimmun Anti Zika Virus ELISA IgM/IgG/IgA kits).

**RESULTS**

Seven hundred and sixty-six samples from patients with a relevant travel history and exposure risk were tested, most of whom were asymptomatic. Fourteen patients were classified as recent infection based upon detection of ZV RNA and/or serological evidence (the presence of IgM or seroconversion to ZV IgG). All patients had travelled from South or Central America. The cohort, 8 males and 6 females, aged 22 to 54 years, had samples collected between days 2 and 35 post-onset of symptoms. ZV RNA was detected in 11/14 (79%); IgM was observed in 8/14 (57%) and IgG in 12/14 (86%). Based upon retrospective analysis, IgA was also detected in 8/14 patients, with one additional sample yielding an equivocal result; Of 9 ZV confirmed patients, antibodies to other flaviviruses were observed in 5 patients. A further 3 patients were ZV IgG positive, IgM/IgA negative. These patients were also serologically positive for other flaviviruses suggesting cross-reactivity. Two children were also born to mothers diagnosed with ZV during pregnancy.

**CONCLUSIONS**

Fourteen returning travellers were identified as recently infected with ZV. Preliminary data indicates that the ZV IgA assay has potential to support the serological diagnosis of recent ZV infection, especially in those individuals with serological evidence of previous flavivirus infection.

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# ABSCENCE OF NOSOCOMIAL TRANSMISSION FROM A CASE OF IMPORTED LASSA FEVER WHEN USING SIMPLE BARRIER NURSING METHODS- A SEROLOGICAL STUDY

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## BACKGROUND-AIM

Nosocomial transmission of Lassavirus is reported to be low when caring for the index patient with proper barrier nursing methods. However, these patients are mostly cared for in high-level isolation units in non-endemic countries which is cost-some, working labour intense, and strenuous for the patient. We have investigated if asymptomatic Lassavirus infection occurred in health care workers who used simple barrier nursing methods during the first 15 days of caring for a patient with Lassa fever in Sweden.

## METHODS

Seventy-six personnel were defined as possible risk of being infected and 53 were included in the study. The included personnel were sampled for blood after a median of 77 days (range 69 – 110) after exposure and the sera were analysed for presence of LASV IgG. They also responded to a detailed questionnaire to evaluate the exposure of different body-fluids from the index patient and the timing of the contacts.

## RESULTS

In none of the samples from the 53 personnel could Lassa virus-specific IgG be detected. Of these, 5 personnel reported fever in conjunction with symptoms possibly suggestive of Lassa fever. Five personnel and one medical student reported that they were not wearing gloves when being in close contact or handling specimens from the patient.

## CONCLUSIONS

The present study confirms a low risk of secondary transmission of LASV in humans when proper basic nursing methods are used and the disease manifests with relatively mild symptoms. In addition, the adequate safety level when caring for patients with suspected or confirmed Lassa fever in non-endemic countries should be further discussed.

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# RAPID ONE-STEP RT-LASER PCR® DETECTION OF EBOLA VIRUS: ANALYTICAL PERFORMANCE EVALUATION AND COMPARISON WITH THE COMMERCIALLY AVAILABLE RT-PCR KIT REALSTAR® FILOVIRUSSCREEN FROM ALTONA DIAGNOSTICS GMBH

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## BACKGROUND-AIM

During the recent Ebola outbreak in West Africa, sensitive and rapid diagnostic methods were highly needed at treatment centers admission points, in order to promptly isolate infected patients. Recently, a new outbreak in the Democratic Republic of the Congo was reported, bringing once more the attention to the need of improved diagnostics. Within the IMI 2-funded consortium FILODIAG (grant agreement No 115844), we are currently developing an ultra-fast molecular amplification assay based on locally laser-heated gold nanoparticles (Laser PCR®), combined with hydrolysis probes for real-time detection. Laser PCR® allows approximately one million times faster temperature ramps than conventional PCRs. We show here the results from a preliminary evaluation of this novel technology.

## METHODS

RNA from a negative human plasma spiked with Makona isolate, was purified (QIAmp Viral RNA Mini Kit, Qiagen) and quantified using the reference test RealStar® FilovirusScreen Kit1.0 RT-PCR kit (Altona Diagnostics GmbH, Hamburg, Germany). Ten replicates of RNA serial dilutions were amplified by Laser PCR® on the prototype instrument Pharos400, and the results were compared with those of the reference test. Additionally, a panel of RNA genomes isolated from 14 different relevant pathogens were tested for specificity evaluation.

## RESULTS

Probit analysis shows that within a runtime of about 35 minutes, the Laser PCR® assay is much faster than the reference test reaching a limit of detection (LoD) of  $2.57 \times 10^3$  cp/ml, which is highly comparable to the LoD of the reference test  $1.39 \times 10^3$  cp/ml. No false positive results were found when targeting water, RNA from healthy donors' plasma, and from a variety of pathogens.

## CONCLUSIONS

Preliminary evaluation of the One-Step RT-Laser PCR® Ebola Assay shows with 100% specificity the ability to detect a very low number of viral copies, and an LoD comparable to the reference test FilovirusScreen Kit1.0. The validation of the assay on samples from Ebola-infected patients from the 2014-2015 outbreak will be carried out next. If the achievements obtained in this preliminary evaluation study will be confirmed by the validation with patients samples, the Laser PCR® assay will prove to be an ideal rapid diagnostic test for Ebola, to be potentially used in future outbreaks.

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### HIGHLY SPECIFIC DIAGNOSIS OF DENV 1-4 INFECTIONS BY ANALYSIS OF ANTI-DENGUE NON-STRUCTURAL PROTEIN 1 IGG ANTIBODIES

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#### BACKGROUND-AIM

Four Dengue virus serotypes (DENV 1-4) exist causing a febrile illness with potentially severe symptoms in humans. After a viraemic phase, in which viral non-structural protein 1 (NS1) is detectable in blood, only antibody determination is effective for laboratory diagnosis. However, a high cross reactivity of the antibodies with other flaviviruses exists, mainly due to the highly conserved viral glycoprotein E. Therefore, we now have relied on the more species specific NS1 antigen in ELISA development.

#### METHODS

The study included 1-2 follow-up samples (n=19) taken within 3-196 days after initial sampling (day 0) of 15 DENV-infected patients, confirmed by positive NS1 antigen test (BioRad) at day 0. Additionally, 190 healthy individuals and 19 patients with previous contact (infection or vaccination) to West Nile (WNV), Tick-borne encephalitis (TBEV) and Yellow fever virus (YFV) were examined.

Samples were analyzed for anti-DENV (1-4) NS1 IgG by ELISAs for which recombinant NS1 antigen of DENV 1-4 were separately expressed in HEK293 cells, purified and coated on microtiter plates. Moreover, a lysate-based ELISA (Euroimmun) for determination of anti-DENV IgG and an ELISA for measuring DENV NS1 antigen (BioRad) were applied according to manufacturer's instruction.

#### RESULTS

10 of 19 follow-up samples (days 3-13) were still positive for NS1 antigen, 9 (days 14-196) were negative. In the anti-DENV (1-4) NS1 IgG ELISAs, 30% of the NS1 antigen-positive, and 100% of the NS1 antigen-negative samples showed positive reactions, in all cases being strongest for the respective infecting serotype.

Specificities for the different anti-DENV (1-4) NS1 IgG ELISAs were 97-99% with respect to healthy individuals. Samples of patients with other flavivirus infections or vaccinations cross reacted with the NS1 antigen in 17-50% (WNV) and 0% (TBEV, YFV) of the cases. In comparison, cross-reactivities in the lysate-based ELISA were 100% (WNV), 50% (TBEV) and 0% (YFV).

#### CONCLUSIONS

The species specific NS1 antigen of DENV 1-4 used for coating ELISA plates provided 100% detection of DENV-infected patients who have passed the viraemic phase (NS1 antigen negative) and reduced cross-reactivity compared to a lysate-based ELISA. Additionally, the NS1-based ELISAs allow serotype differentiation useful in epidemiological studies.

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### HUMAN MACROPHAGES DIFFERENTIATED IN THE PRESENCE OF VITAMIN D RESTRICT DENV INFECTION BY MODULATING MANNOSE RECEPTOR EXPRESSION

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#### BACKGROUND-AIM

Severe dengue disease has been associated with high viral loads and high-altered production of pro-inflammatory cytokines, suggesting impairment in the control of dengue virus (DENV) and the negative feedback mechanisms that regulate cytokine production. Interestingly, vitamin D has been described as an important modulator of immune responses to several pathogens. There is increasing evidence that vitamin D status and activity is associated with less DENV infection and early disease recovery, however, the molecular mechanisms by which vitamin D reduces DENV infection are not well understood. Our aim was examined the antiviral effect of vitamin D on Dengue virus (DENV) replication and cytokine production in DENV-infected monocyte-derived macrophages (MDM).

#### METHODS

MDMs differentiated in the presence or absence of vitamin D was infected with DENV; the mannose receptor and DENV RNA replication were examined by quantitative PCR. The antiviral effect of vitamin D on DENV replication was determined by measurement of virus load by flow cytometry and cytokines production by ELISA.

#### RESULTS

We observed that MDMs differentiated in presence of vitamin D (D3-MDM) are less susceptible to DENV infection likely due to downregulation of some C-type lectins such as mannose receptor (MR, CD206) a key target molecule for attachment of DENV to macrophages and an important trigger of DENV-induced cytokine responses. In addition to low MR levels, also less binding of DENV to macrophages and less DENV-induced production of TNF- $\alpha$ , IL-1, and IL-10 in D3-MDM was observed.

#### CONCLUSIONS

Collectively, these findings indicated that vitamin D affect DENV replication in D3-MDM and decreases the inflammatory response to viral infections, suggesting a possible molecular mechanism by which vitamin D can contribute with DENV control and progression of severe disease.

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# CAN ZIKA VIRUS BE REMOVED FROM SEMEN BY PERFORMING A WASH PROCEDURE PRIOR TO USE IN ASSISTED REPRODUCTION SETTING?

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## BACKGROUND-AIM

Zika virus (ZIKV) infections during pregnancy are associated with adverse fetal outcomes. Also persistent shedding of ZIKV RNA in semen after acute infection is demonstrated. The combination of its known teratogenicity and the presence of the virus in semen, makes it necessary to review protocols for assisted reproductive technologies (ART) when patients return from ZIKV endemic areas. Because of the potential transmission of ZIKV, we explored the possibility to remove the virus with ZIKV spiked semen samples.

## METHODS

Six semen samples were spiked with ZIKV culture supernatant that was isolated from a semen sample originating from a ZIKV infected patient from Trinidad. ZIKV culture supernatant was added at clinically relevant viral loads. In order to remove the virus, the spiked samples were processed by a separation and a washing procedure based on Savasi et al., 2013. This procedure is routinely used for the processing of semen samples of HCV and HIV serodiscordant couples. In brief, the semen sample was first separated by a density gradient centrifugation and subsequently washed twice. An in-house developed real-time RT-PCR for the detection of ZIKV RNA was performed on all spiked samples prior and after the procedure. Virus isolation on Vero cells was also performed on each sample. The viral growth was monitored by checking the cells daily until day 7 for appearance of cytopathic effects (CPE).

## RESULTS

Regarding RT-PCR results, Ct-values before washing ranged from 19 to 29. After the procedure, viral RNA was still detectable in 5/6 samples with Ct-values ranging from 35 to 44. ZIKV was successfully isolated by viral culture from all the unprocessed samples. The washing procedure had a clear influence on viral culture outcome: only 1/6 samples showed CPE after the procedure.

## CONCLUSIONS

The washing procedure used is insufficient to remove all ZIKV RNA from spiked semen samples. However, based on the results of viral culture, there is a possibility that this procedure may be sufficient for semen samples with lower viral loads (Ct >30), but further investigation is required. In a next step the procedure should be tested on ZIKV positive semen samples of infected men.

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# SEROEPIDEMIOLOGY OF VECTOR-BORNE VIRAL INFECTIONS (ALPHA- AND BUNYAVIRUSES) IN IRAQ

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## BACKGROUND-AIM

Despite its significance and increasing public health importance in human globally, viral zoonoses still poorly understood and not documented in Iraq. The study designed to investigate the seroprevalence of selected vector borne viruses from the group of Alphaviruses (Sindbis and Chikungunya viruses) and Orthobunyaviruses (Batai, Inkoo and Sathuperi viruses) in Iraq in relation to the clinical presentation and geographic locations of the study area.

## METHODS

Blood samples were collected from three groups of individuals (patients with acute febrile illness, symptomatic patients with associated underlying diseases and healthy individuals) with a total number of 485 from five locations at Al-Nasiriyah Governorate. Direct immunofluorescence (IFA-IgG) was used to detect virus-specific antibodies on cell-cultures (Vero E6 cell line) infected with the above viruses.

## RESULTS

The overall seroprevalence to Alphaviruses was 3.5% (Sindbis 2.5% and Chikungunya virus, 1.03%) and Orthobunyaviruses was detected in 0.82% (Batai virus 0%, Inkoo virus 0.6% and Sathuperi virus 0.2%). Sindbis virus seropositivity was higher (4.5%) among patients with associated underlying diseases (Risk group) compared to febrile patients (1.1%) and healthy individuals (1.9%) and more in rural (2.8%) than in urban (1.8%). A similar pattern was observed to Chikungunya virus. For orthobunyaviruses, Inkoo virus was detected in 2% of patients with underlying diseases and Sathuperi virus in 0.6% only and Batai virus not detected in the area among any patient group although they have a worldwide distribution.

## CONCLUSIONS

Alphavirus group is prevalent in our area (3.5%) with varying presence for their members. Orthobunyaviruses group are present to a less extent (0.82%) with varying percentages for their members (Inkoo and Sathuperi virus) with complete absence of Batai virus in this group. These new viruses circulating in our area especially among patients with associated chronic diseases. The results of this study implies the consideration of these viruses in clinical practices.

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**MOSQUITO-BORNE VIRUSES IN SOUTHERN AND COASTAL KENYA**

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**BACKGROUND-AIM**

Many mosquito-borne viruses (MBVs) originate in tropical Africa, where there is both high wildlife reservoir and vector species diversity in close vicinity of human habitation. In Africa, the lack of resources for specific laboratory diagnostics is likely to result in underestimates of the variety of MBVs and their impact to public health. Additionally, the number of detected human cases is often a fraction of those transmitted; therefore, surveys of wildlife (vectors and potential vertebrate hosts) are needed to obtain reliable distribution data for MBVs. This study is funded from Academy of Finland development research targeted to study dengue virus in endemic areas, especially in Africa in collaboration between University of Helsinki and University of Nairobi. The fieldwork has been performed from the University of Helsinki research station in Taita Hills.

We aim to obtain information on the prevalence and diversity of dengue and other MBVs and mosquito species transmitting these viruses in southern and coastal Kenya. We also aim to predict the risk areas for MBV transmission.

**METHODS**

We have already collected mosquitoes (n=15000) and human samples from febrile patients, including sera (n=500) and urine (n=330) samples, from southern and coastal regions of Kenya in years 2015-2017 and the collections are still ongoing. The mosquito material species identification is done using morphological and genetic analyses.

Human and mosquito samples are screened for viral nucleic acids and human samples also for antibodies against MBVs such as dengue (DENV) and chikungunya virus (CHIKV). Virus isolation in cell cultures and NGS methods will be used to detect and characterize viruses in human and mosquito samples.

**RESULTS**

The laboratory analysis of the human and mosquito specimens are currently ongoing. Of the so far tested human sera, 13% were found positive for DENV IgG (IFA) and 0,5% for DENV IgM (ELISA). Additionally, 6% of the tested sera have been found positive for CHIKV IgG, the IgM testing is ongoing. The determination of virus species present in the region requires either determination of antibody specificity by neutralization tests or confirmation by viral genomic sequence data.

**CONCLUSIONS**

The preliminary results suggest circulation of flavi- and alphaviruses in southern regions of Kenya.

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**SURVEILLANCE OF HEPATITIS A VIRUS IN PORTUGUESE INFLUENT AND TREATED EFFLUENT WASTEWATERS: ARE CURRENTLY USED TREATMENT EFFECTIVE ENOUGH?**

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**BACKGROUND-AIM**

Hepatitis A virus (HAV), represents one of the main waterborne and foodborne pathogens associated with hepatitis outbreaks worldwide. Europe is currently seeing an outbreak of HAV, mainly linked to men who have sex with men. Despite the transmission mode, human infection will result in the introduction of high quantity of virus in wastewater systems, requiring powerful treatment processes in order to efficiently remove the virus and prevent its discharge in aquatic environment, and further dissemination through human population. The lack of data on the presence of HAV in Portuguese wastewater systems prompted to this study.

**METHODS**

HAV occurrence was evaluated in sixty Portuguese influent and respective effluent wastewaters samples, collected all across the country, in autumn and winter of 2013. After virus concentration, all samples were submitted to nucleic-acid extraction followed by real time RT-PCR, using primers and TaqMan probe specific for HAV genome.

**RESULTS**

Only one wastewater treatment plant, located in north of Portugal, revealed the presence of HAV genome. HAV was detected in both influent and effluent samples. These results point to the fact that despite HAV is not frequent in Portuguese wastewaters, it may have circulated in that specific region of the country, probably associated with asymptomatic infection, since no outbreak was noticed during such period. The further detection of viral genome in the respective effluent sample points to the ineffectiveness of the used treatment to efficiently remove HAV from wastewater.

**CONCLUSIONS**

The present study represents the first assessment of HAV in Portuguese wastewaters, and apart from the low frequency of HAV detection, claim for attention to the used treatment processes. Commonly used processes may not be effective enough to remove viruses from wastewater, as occur with HAV, and represent a source of discharge of this organisms into water, spreading them to soil and food, and ultimately to humans.

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# **SENSITIVE AND RELIABLE DETECTION OF ZIKA VIRUS WITH THE CLONIT QUANTY ZIKA RT-PCR TEST USING VERSANT MOLECULAR SP**

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## **BACKGROUND-AIM**

Zika virus (ZIKV) is a flavivirus first isolated in 1947 in Uganda that is transmitted through the bite of an Aedes mosquito.<sup>1</sup> Infection with ZIKV during pregnancy is associated with fetal microcephaly and other serious birth defects.<sup>2</sup> The Clonit quanty Zika RT-PCR Assay<sup>3</sup> (Clonit, Milan, Italy) is a real-time-PCR based in vitro diagnostic test intended for quantitative detection of Zika virus RNA in clinical specimens (Serum, Urine, and Saliva).<sup>4</sup> The Clonit test contains reagents for reverse transcription and specific amplification of the NS5 region of Zika Virus RNA. In addition, the assay has a built-in standard curve for quantitation and extraction control to confirm the validity of the extraction process. In this study, we compare the performance of the Clonit Zika RT-PCR Assay with an assay from Lanciotti et al (2008).

## **METHODS**

Zika RNA was extracted from serum and urine samples with the VERSANT® kPCR Sample Prep5 and VERSANT Sample Preparation 1.0 reagents and then amplified on the Thermo Fisher QuantStudio 5 thermal cycler. A comparison with an assay using primers/probe from Lanciotti et al was conducted on 42 paired serum and urine specimens, previously confirmed PCR-positive, or suspected positive by a physician.

## **RESULTS**

The Clonit Zika assay detected 17 positive specimens (80%) and the Lanciotti assay detected 18 positive specimens (85%) among the 21 serum specimens. The Clonit Zika assay detected 16 positive specimens (76%) and the Lanciotti assay detected 13 positive specimens (13%) among the 21 urine specimens.

## **CONCLUSIONS**

The data demonstrates that the Clonit Zika assay results are comparable in serum and more sensitive in urine when compared to the CDC. Clinical specificity testing is in progress.

1. WHO, Fact Sheet, 2016
2. One-step RT-PCR for detection of Zika virus; Oumar Faye, Ousmane Faye, Anne Dupressoir, Manfred Weidmann, Mady Ndiayed, Amadou Alpha Sall
3. CE-marked for IVD use.
4. Clonit quanty Zika RT-PCR Assay Product Insert
5. VERSANT is a registered trademark of Siemens Healthcare Diagnostics Inc. All other trademarks and brands are the property of their respective owners. Product availability may vary by country.

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# **VALIDATION OF A MOLECULAR IN-HOUSE METHOD FOR THE DETECTION OF ZIKA VIRUS RNA IN HUMAN SPECIMENS**

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## **BACKGROUND-AIM**

Due to the increase of outbreaks in Africa, America, Asia and the Pacific region since 2015 and its presumptive association with severe congenital brain abnormalities, the Zika virus has been declared a Public Health Emergency of International Concern (PHEIC). For this reason, reference virology laboratories need suitable test methods for laboratory confirmation of Zika virus infections.

Aim of this study was to determine if the developed in-house real-time PCR is appropriate for the detection of Zika virus in human samples and to validate the real time PCR method for the detection of Zika virus RNA according to the accreditation norm ISO 15189:2012.

## **METHODS**

The different statistical parameters were calculated using the ct-values of 9 different runs to prove the quality of the obtained results.

## **RESULTS**

The limit of detection observed was  $4.2 \times 10^3$  copies/ml (152 copies/reaction). In addition, the calculated trueness (100 %), accuracy (1), precision (coefficient of variation 0.33 % - 4.75 %), selectivity (100 %), and sensitivity (100 %) showed excellent results. Serum and urine were identified as suitable specimens. The regression coefficients calculated on 2 dilution series are with 0.9953 and 0.9882 higher than the required limit (0.98), the PCR-efficiency (82.85 % respectively 81.57 %) is below the 90 % threshold required for a quantitative method, but quantification of the viral load has never been the aim of this study. The sequence analysis of the amplicons revealed significant alignments only with Zika virus. A cross reactivity with TBE virus, West Nile virus, Yellow Fever virus and Dengue virus could be excluded by testing potentially cross-reactive samples. The measurement uncertainty of the method is 1.43, which is not exceeding the intrinsic variability of the methodology used and refers to the ct-value of the reaction. The determination of diagnostic parameters - carried out using the available samples - showed excellent results.

## **CONCLUSIONS**

Overall, the validated in-house method can be used for the detection of the viral pathogen ZIKV in biological specimens from patients with a potential active Zika virus infection.

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# EXTERNAL QUALITY ASSESSMENT TO EVALUATE MERS CORONAVIRUS AND ZIKA VIRUS MOLECULAR TESTS PERFORMED BY NONGOVERNMENTAL CLINICAL LABORATORIES

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## BACKGROUND-AIM

In order to prepare for the emergence of MERS and Zika, MERS coronavirus (MERS-CoV) and Zika virus molecular tests have been extended to nongovernmental medical institutions since August 2016. An external quality assessment (EOA) was needed to assess the quality of the assays performed by the participating laboratories.

## METHODS

The first was a survey of the participating laboratories' practices, focusing on testing volume and the reagents used. The second project was the production of external quality control materials for molecular testing of MERS-CoV and Zika virus, as well as an appraisal of the proficiency of MERS-CoV and Zika virus molecular tests performed by nongovernmental medical laboratories. The third task was the "Quality Improvement Workshop for Molecular Tests of MERS-CoV and Zika Virus."

## RESULTS

A survey of 37 participating laboratories showed a high response rate of 97.3%, and it was suggested that a rapid EOA was necessary to assess the quality of the assay used. Twenty-five laboratories participated in the MERS-CoV EOA, and 33 laboratories participated in the Zika virus EOA. The MERS-CoV EOA panel of 12 samples, and the Zika virus EOA panel consisted of 5 samples. The MERS-CoV EOA results were 100% correct for all tested samples, however, one laboratory's results showed "outliers." The fact that one laboratory reported false positive Zika virus results ("unacceptable") in a negative sample shows that room for improvement still exists. In the homogeneity and stability test of the external quality control materials, both MERS-CoV and Zika virus quality control materials showed good homogeneity and stability up to 72 hours at 4°C and 8 weeks at -70°C.

## CONCLUSIONS

The study identified problems stemming from the introduction of the emergency use approval system and from the experimental expansion of MERS and Zika testing to nongovernmental medical institutions since August 2016. In addition, the reliability of MERS-CoV and Zika virus molecular testing performed by nongovernmental laboratories was evaluated. In the future, this study will help to establish a laboratory response system for emerging and re-emerging viral diseases, and will serve as a basis for strengthening civilian capacity in the diagnosis of emerging and re-emerging viral diseases.

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# INVESTIGATION OF ZIKAVIRUS KNOWLEDGE AMONG PHARMACY STUDENTS IN EASTERN MEDITERRANEAN UNIVERSITY, TURKISH REPUBLIC OF NORTHERN CYPRUS

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## BACKGROUND-AIM

To investigate Zikavirus knowledge of pharmacy students and compare the level of the knowledge according to their native countries.

## METHODS

The questionnaire modified from World Health Organization was performed. Total number of respondents was determined to be 216, regarding total number of students registered to the faculty as 1120, confidence level 95% and confidence interval 6%. Number of students according to native country was determined by stratified random sampling. Students who have not heard about Zika (first question) were excluded. The statistical analysis of the data was evaluated by IBM statistical program for social sciences (SPSS) version 21.

## RESULTS

Of 226 students, 144 (64%) were female and 82 (36%) were male. 151 (67%) of them heard about Zika. 49 female and 26 male participants have never heard about Zika. No statistically significant difference was observed between gender and the awareness ( $p>0.05$ ).

Of 151 students; 41, 35, 28, 17, 10 and 7 were Nigerian, Iranian, Turkish Cypriot, Syrian, Iraqi and Turkish, respectively, and 13 were from other countries. 77%, 76%, 71%, 61%, 50%, 48% of Syrian, Nigerian, Iranian, Turkish Cypriot, Turkish and Iraqi students heard about Zika, respectively ( $p>0.05$ ).

Approximately 60% of students heard about Zika in 2015. Television/radio and internet/social media were the most frequently reported means of being aware of the disease.

Aetiological agent was reported to be a virus by 77% of participants; followed by parasite (12%) and bacteria (3%). 8% of students did not give any answer. 65% had no idea about the incubation period.

Mosquito bite was reported as the transmission mode by 77% of students, where as mother to child transmission by 32%, sexual intercourse by 20% and blood transfusion by 19%.

71% of Nigerian, 40% of Iraqi, 36% of Turkish Cypriot, 31% of Iranian, 29% of Turkish and 18% of Syrian students heard about Zika outbreak. The difference between Nigerian and Iranian and Nigerian and Syrian students for being aware of Zika outbreak was statistically significant ( $p=0.0029$  and  $p=0.0046$ , respectively). 46% of students had no idea about the treatment.

## CONCLUSIONS

Zika outbreak in 2015 increased the awareness of pharmacy students about Zika. The knowledge about incubation period and treatment were inadequate.

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# **EVALUATION OF DENGUE VIRUS (DENV)-SPECIFIC T-CELL EFFECTOR MEMORY RESPONSE BY EX-VIVO IFN- $\gamma$ ENZYME-LINKED IMMUNOSPOT (ELISPOT) ASSAY**

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## **BACKGROUND-AIM**

Dengue virus (DENV) infection has dramatically grown in recent years becoming one of the major emerging infectious diseases. DENV is caused by four serotypes (DENV-1-4), transmitted by *Aedes* spp. mosquitoes. Due to the highly cross-reactive immune responses between DENV serotypes and other arthropod borne Flavivirus, study the DENV-specific T-cell response in subjects who experienced more than one infection might add useful information.

## **METHODS**

A novel ex-vivo ELISPOT assay for the evaluation of DENV-specific T-cell response was developed and tested in 11 DENV-seropositive and 6 ZIKA-seropositive subjects. As controls, 20 DENV and ZIKA seronegative healthy subjects were enrolled. Lymphocytes were stimulated with peptide pools, 15-18 aminoacids in length with 11 overlapping, allowing the stimulation of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, representative of the whole protein NS3 of DENV 1, 2, 3 and 4 at the final concentration of 1  $\mu$ g/mL. IFN secreting T-cells were detected by ex-vivo ELISPOT assay and results were given as net spots/million PBMC.

## **RESULTS**

The mean NS3 DENV-specific response plus 2 times the SD of the DENV-seronegative subjects was used to set a threshold above which T-cell response was considered positive (30 net spots/million PBMC). DENV-specific T effector response was detectable in 11/11 DENV-seropositive subjects [median (IQR) D1, D2, D3 and D4-specific T-cell response respectively 133 (70.5-208.5), 138 (57.5-495), 125.5 (58.5-284.3) and 116.5 (48-353) net spots/million PBMC;  $p < 0.0001$ ]. Sensitivity and specificity of the assay were respectively 91.7% and 95% with NPV 87.5% and PPV 95%. The 6 patients who developed only ZIKA infection did not shown DENV-specific T-cell response. In these patients medians D1, D2, D3 and D4-specific T-cell response were respectively 6.5 (IQR 3.75-21.25), 8 (IQR 5-16), 5.5 (IQR 0-15.75), 10 (IQR 8-17.5) net spots/million PBMC. Furthermore a correlation between T-cell response and neutralization assay for all DENV serotypes was documented.

## **CONCLUSIONS**

A novel DENV-specific ELISPOT assay has been developed which might be useful to investigate DENV-specific T-cell response and improve ZIKA diagnostics in patients with preexisting DENV immunity.

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# **THE IMPACT OF PRE- AND WEEK 2 AND WEEK 4 POST-TRANSPLANT CMV-SPECIFIC ELISPOT ASSAY ON CMV REACTIVATION IN CMV-SEROPOSITIVE ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (ALLO-HCT) RECIPIENTS**

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## **BACKGROUND-AIM**

CMV infection causes significant morbidity and mortality in allo-HCT. CMV cell-mediated immunity (CMI), assessed by engrafted T cell production of IFN $\gamma$ , is a major mechanism to control CMV replication. The potential role of pre- and post-transplant CMI and its impact on CMV replication and survival is not well understood.

## **METHODS**

This is a multi-center (13 sites), prospective, observational study of adult CMV seropositive allo-HCT recipients. T cell responses were serially monitored pre-transplant (screening), and every 2 weeks post-HCT up to 26 weeks with an ELISPOT assay that uses CMV-specific IE-1 and pp65 antigens (T-SPOT. CMV, Oxford Diagnostics Laboratories®, Memphis, TN). The changes in spot counts (SPCs) at 4 weeks post-HCT from screening for both antigens and its impact on first CMV reactivation were assessed. Additionally, week 2 pp65 >100 was correlated with the occurrence of first CMV reactivation.

## **RESULTS**

Of 244 enrolled patients, 236 patients had a week 2 visit while 151 subjects had both a screening and week 4 visit. Majority of the 244 enrolled patients are white (73%), males (56%), and median age of 56 years (22 – 80). More patients (46%) had unrelated and 36% had matched HCT, and 55% had a CMV seropositive donor. Changes in SPCs between screening and week 4 post-HCT were associated with first CMV reactivation occurring post-week 4 were assessed. Using a positive change for SPCs for both IE1 and pp65 (SPCs at week 4 > SPCs pre-transplant), a negative predictive value (NPV) for the development of first CMV reactivation was 80.0% and 83.7%, respectively. The NPV for the development of CMV reactivation at week 2 and week 4 pp65 >100 were 92.0% and 91.3%, respectively, during the 26 week study period (Figure).

## **CONCLUSIONS**

Assessment of CMV-specific CMI at screening and 4 weeks post-HCT may prove useful for determining the likelihood of protection against CMV reactivation. Additionally, immune monitoring at 2 and 4 weeks post-transplant demonstrated a NPV for the development of the CMV reactivation throughout the course of the study of 92% and 91.3%, respectively. These data suggest the utility of a single measurement but need further validation.

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### ROLE OF PATTERN RECOGNITION RECEPTORS IN THE PATHOGENESIS OF HERPES SIMPLEX VIRUS -1 INDUCED UVEITIS IN A RABBIT ANIMAL MODEL

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#### BACKGROUND-AIM

HSV-1 plays an important role in the causation of viral anterior uveitis. Among the various host factors responsible for pathogenesis, pattern recognition receptors (PRRs) play an important role. The present study aimed to study the role of PRRs in the pathogenesis of HSV-1 induced uveitis in a rabbit animal model.

#### METHODS

A rabbit animal model of HSV-1 was developed by inoculation of HSV-1 in the anterior chamber of the rabbit eye (N=9). The ocular signs were monitored daily by slit lamp biomicroscopy. Three rabbits each were sacrificed on day 1, day 3 and day 5 post infection. The mRNA expression of TLRs (2, 3, 4, 9), RIG-1, MDA5, NOD2, TNF, IL- (1, 4, 6, 8, 10, 12A, 17A, 23A), IFN, INF and STING were studied and also histopathology was performed. The HSV-1 DNA load was estimated in the aqueous humour.

#### RESULTS

The clinical evaluation of eyes showed the presence of keratic precipitates, cells and flares with the maximum cells (3+) and flares (4+) being observed on day 1 p.i. Up regulation was observed in majority of PRRs studied with the highest fold change expression seen in TLR 2, RIG-1, MDA5 and STING (>10 fold, p<0.05). Among the interferons, INF was found to be up regulated (30 fold). A decline in HSV-1 load was observed from day 1 (1129 copies/μl) to day 5 (226 copies/μl) p.i. The up regulation (>10 fold, p<0.05) of TNF, IL-(1, 6, 8, 12A, 17A and 23A) might be responsible for inflammatory process in uveitis and in recruitment of immune cells. Also, biphasic up regulation of IL6, IL17A and IL23A was observed at day 1 (approx. fold change 3000; 900 and 60) and at day 5 p.i. (approx. fold change 400; 10 and 15) respectively. The histological examination of eyes showed severe inflammation on day 1 and day 5 p.i. The predominant response in ciliary body was neutrophilic on day 1 p.i, while lymphocyte and macrophages infiltration were observed on day 5 p.i. The retina was found to be spared from inflammatory response.

#### CONCLUSIONS

In HSV-1 induced anterior uveitis, PRRs particularly TLR2, RIG-1, MDA5, possibly modulate various inflammatory cytokines and activates innate antiviral immune response. Further, IL6, IL17A, and IL23A play an important role in causing inflammation and hence may be potential therapeutic targets

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### COMMERCIAL INTRAVENOUS IMMUNOGLOBULINS INDUCE HIGH BKV GENOTYPE-SPECIFIC NEUTRALIZING ANTIBODY TITERS IN KIDNEY TRANSPLANT RECIPIENTS.

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#### BACKGROUND-AIM

BK virus-associated nephropathy (BKVAN), a consequence of the strong immunosuppressive therapy given after kidney transplantation (KT), represents a growing medical problem in the KT setting. At present, there are no BKV-specific antiviral therapies available. In a previous study, we have demonstrated that BKV genotype-specific neutralizing antibodies (NAbs) play a key role in protection against BKV replication after transplantation (Solis et al, ESCV 2016), supporting the potential benefit of administering NAbs as a preventive strategy against BKV infection.

#### METHODS

In the present study, we evaluated the capacity of 3 commercial intravenous immunoglobulins (IVIg) (Privigen®, Clayrig®, Octagam®) to neutralize the three major BKV genotypes in vitro using the BKV pseudovirion system and BKV virions isolated from KTR with BKVAN. We further evaluated BKV NAb titers in plasma samples of KTR after administration of IVIg for acute antibody-mediated rejection (1g/kg/day, 1 cure/week for 3 weeks, n= 18 patients) or for secondary immunodeficiency syndrome (0.4 g/kg/day, one cure, n=11 patients). Plasma samples were collected before and 24 hours after each administration of IVIg, and three months after the last administration.

#### RESULTS

In vitro, IVIg preparations show a high and reproducible anti-BKV neutralizing activity with NAb titers ranging from 5.04 to 6.68 log10, 4.02 to 5.51 log10, and 3.59 to 5.14 log10 against genotype I, II, and IV, respectively. In vivo, all patients show an increase of NAb titers in plasma after IVIg administration by 60 fold for genotype I to 300 fold for genotype II and IV. Patients harboring NAb titers lower than 4 log10 were able to reach high NAb titers against the three major BKV genotypes (3.8 to 5.48 log10). The high NAb titers persist 3 months after the last IVIg administration. Interestingly, we found similar NAb titer increase in patients treated by 1g/kg/day compared to those treated by 0.4g/kg/day, for the three genotypes.

#### CONCLUSIONS

These data demonstrate that IVIGs have an important anti-BKV neutralizing activity. Our results provide the rationale for a proof-of-concept study investigating the efficacy of IVIg for prevention of BKV replication after transplantation.

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# IMPACT OF IFN LAMBA III/4 SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ON THE CYTOMEGALOVIRUS (CMV) REACTIVATION IN AUTOLOGOUS STEM CELL TRANSPLANT (AUTO-SCT) PATIENTS

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## BACKGROUND-AIM

CMV infection represents one of the main cause of morbidity and mortality after SCT. Type III interferons (IFNs), including IFN1 (IL29), IFN2 (IL28A) and IFN3 (IL28B) are thought to display potent antiviral and immunomodulatory properties in vivo. Rs12979860 SNP in IL28B gene region is located 367 bp from the functional dinucleotide variant rs368234815 (IFN4-TT/G) and is known to influence the spontaneous and treatment-induced clearance in HCV infection. Recently, a protective effect of the T allele of rs12979860 against CMV infection in the Allogenic stem cell transplantation was suggested. The present study was aimed at investigating whether the rs12979860 (IL28B) and rs368234815 (IFN4) SNPs might effect the incidence of active CMV infection in the Auto-SCT.

## METHODS

The study included 99 patients (Median 56 years) who underwent Auto-SCT. The SNPs genotype was determined by Real Time PCR followed by Melting analysis. CMV DNA levels were monitored by quantitative Real-Time PCR (PLX CMV, KPCR, Siemens) weekly for 3 months after the infusion.

## RESULTS

Data from SNPs analysis showed the following rs12979860 SNP genotype distribution between patients: CC 46.5%, CT 41.4%, TT 2.1%. The Minor allele frequency of the T allele was 0.3 and perfectly overlaps to the one observed in general population. CMV reactivation was documented in 50% (TT), 35% (CC) and 29.2% (CT) of patients. Rs12979860 TT genotype exactly overlapped the presence of G/G IFN4. No differences in CMV copies number were recorded at reactivation even though patients carrying CC/CT genotype tends to have a lower rate of reactivation (32% vs 50%) and an higher proportion of asymptomatic reactivation (65% vs 17%) when compared to TT patients. The analysis of patients older than 60 years showed a significantly higher incidence of active CMV infection in the TT subjects (83%) with respect to CC (21%) and CT (40%) carriers. Multivariate analysis suggested an independent predictive role of NHL, type of conditioning regimen and concomitant bacteria infections on CMV reactivation (p>0,05).

## CONCLUSIONS

Our data suggest a protective role of rs12979860 CC/CT genotype in the reactivation of CMV infection especially in older patients along with other clinical data and may be relevant in the context of monitoring CMV infection in Auto-SCT.

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# CYTOTOXIC TRAIL+ NK CELLS OVERCOME HCV INHIBITION AND MASTER ANTI-VIRAL IMMUNITY

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## BACKGROUND-AIM

Natural Killer (NK) cells respond to HCV infection by releasing pro-inflammatory anti-viral cytokines (IFN $\gamma$ , TNF $\alpha$ ) or by cell-to-cell contact using TRAIL-mediated apoptosis. HCV infection is highly persistent as virus inhibits host's immune system and NK cell function. Aim of this study was to discover if response to HCV infection can be improved by differentiating NK cells in vitro with selected cytokines.

## METHODS

Healthy donors' NK cells were differentiated with a selected mix of cytokines into cytotoxic TRAIL+ or cytokine-releasing IFN $\gamma$ +TNF $\alpha$ +NK cells and phenotype assessed by Flow Cytometry. Cytotoxicity was determined by CRA. NK anti-viral function was studied in transwell co-cultures with HCV-infected Huh7.5 cells; infection was traced by immunofluorescence and HCV Core Ag quantification. miRNome signature was investigated by NGS and cytokine profiling by Multiplex and Mass Spectrometry.

## RESULTS

NK cell differentiation with any of the cytokine cocktails tested induced upregulation of NKp30, NKp44 and NKp46. By cell-cell contact, cytotoxic TRAIL+ NK cells killed with the highest efficiency HCV-infected target cells, while cytokine-releasing IFN $\gamma$ +TNF $\alpha$ +NK were less functional. Surprisingly, transwell co-culture assays demonstrated that TRAIL+ NK cells consistently and fully eradicated HCV infection by releasing soluble factors, while IFN $\gamma$ +TNF $\alpha$ +NK failed to clear the virus. By comparative miRNome analysis we underpinned mir-181a-3p and by cytokine profiling we identified the protein MX-1. Notably, hierarchical clustering also showed systematic variations in the miRNA expression among the different groups.

## CONCLUSIONS

The pathways identified in activated TRAIL+ NK cells that specifically characterize their enhanced anti-viral function compared to IFN $\gamma$ +TNF $\alpha$ +NK NK described herein might represent a tool to license fully functional NK cells with a reduced sensitivity to viral inhibition.

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**DRY ORAL NYLON FLOQSWABS™ FOR THE DETECTION OF VIRAL ANTIBODIES**S. Castriciano <sup>1</sup>, D. Speicher <sup>2</sup>, K. Luinstra <sup>2</sup>, M. Smieja <sup>2</sup><sup>1</sup>Copan Italia Spa, Brescia, Italy<sup>2</sup>St. Joseph's Healthcare, Hamilton, ON, Canada**BACKGROUND-AIM**

Saliva contains viral antibodies and might be used to monitor seropositivity to aid vaccination and transplantation programs. However, endonucleases are problematic. Dried oral swabs could be a non-invasive and simple alternative to blood sample collection as samples can be easily collected and stored at room temperature. We produced a modified method which allowed dried oral swabs to aid in detecting viral antibodies.

**METHODS**

Study cohort consisted of 50 healthy volunteers (15 males:35 females) with an average age of 43.4 years (range: 18-65 years). From each participant sera, whole saliva, and oral nylon FLOQSwab™ (Copan Italia, Brescia Italy) were collected. Sera and saliva was stored at -80oC FLOQSwab™ were air dried and stored at room temperature. Seroprevalence for Cytomegalovirus (CMV), Varicella Zoster Virus (VZV), Epstein-Barr virus (EBV), measles and mumps IgG antibodies was determined on sera, oral swabs and saliva via commercial ELISA kits (R-Biopharm, Gold Standard Diagnostics) and processed on the ThunderBolt® Analyzer (Gold Standard Diagnostics).

**RESULTS**

For CMV-IgG, as compared to sera, swabs and saliva were 95.8% sensitive/100% specific/96.0% accurate and 96.0% sensitive/93.3% specific/92.9% accurate respectively. All volunteers were 100% seropositive for VZV due to vaccination or natural illness. For VZV, oral swabs and saliva were 96.0% and 93.9% sensitive respectively, and 100% specific for both fluids. Sensitivity of oral swabs and saliva for EBV EBNA-1 and VCA were 92.1% and 91.9%, and 95.5% and 97.7%, respectively. Oral swabs and saliva had poor sensitivity for measles and mumps; 85.4% and 93.9%, and 62.2% and 73.3% respectively. For all viruses studied swabs correlated well with saliva.

**CONCLUSIONS**

Dried oral FLOQSwab™ correlated well for CMV, VZV, and EBV antibodies with excellent sensitivity and specificity, but do not work well to detect antibodies for measles and mumps. FLOQSwab™ are not invasive and easy to collect, can be stored at room temperature, and are an ideal tool for donor immune status screen and for sero-prevalence studies

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**MOLLUSCUM CONTAGIOSUM MHC-I HOMOLOGUE M080 PROMOTE CD8+ T CELL EVASION AND MODULATES NK CELL RECOGNITION BY DOWNREGULATING ENDOGENOUS CLASSICAL MHC-I, HLA-E.**H. Elasifer <sup>1</sup>, J. Bugert <sup>1</sup>, V. Prod'homme <sup>1</sup>, E. Wang <sup>1</sup>, R. Stanton <sup>1</sup>, C. Fielding <sup>1</sup>, R. Aichele <sup>1</sup>, G. Wilkinson <sup>1</sup><sup>1</sup>Cardiff University**BACKGROUND-AIM**

Molluscum contagiosum virus (MCV) is species specific and the only poxvirus endemic in human since global eradication of Smallpox. MCV infects and transforms primary keratinocytes in epidermis. The infected cells will proliferate, but productive infection is not activated until the cells undergo differentiation. During this prolonged replication cycle the virus must resist host immune surveillance. MCV encodes two major histocompatibility complex (MHC) class I (MHC-I) homologues MC0801 and MC033. The aimed: to investigate the effect of MC080 and mc033 on immune surveillance

**METHODS**

Using replication deficient adenovirus to express mc033 and mc080, we examined the effect of mc033 and mc080 on surface expression of MHC-I and MHC HLA-E by using FACS. We also used Western blotting to measure the total expression of MHC-I, and the effect of Endo- H and PNGase. We examined the effect of mc080 on NK cell and CD8+ T cell function

**RESULTS**

When mc080R and mc033L genes were expressed by using a replication-deficient adenovirus vector, MC033 and MC080 were detected as glycoproteins located in the ER. MC080 selectively downregulated cell surface expression of classical MHC-I and the non-classical MHC HLA-E. MC080, but not MC033 prevented HCMV-specific T cells being activated by peptide-pulsed targets. MC080 had variable effects on NK cells activation. We therefore propose that MC080 acts as both a CD8+ T cell and an NK cell evasion function.

**CONCLUSIONS**

MC033 and MC080 are glycoproteins retained in the ER. MC080 downregulates HLA-I in a TAP-independent fashion, also MC080 protects against CD8+ T cells and modulates NK cell activation.

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### ABILITY OF MUMPS VACCINE INDUCED NEUTRALIZING ANTIBODIES TO PROTECT AGAINST MUMPS VIRUS G5 GENOTYPE

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#### BACKGROUND-AIM

Large-scale mumps outbreaks were reported in vaccinated population worldwide. In Israel, we experienced several mumps outbreaks in the past few years.

Since waning immunity has been proposed to be the cause of secondary mumps vaccine failures, revaccination during adolescence was suggested as possible measure to prevent mumps virus reinfection. The decision of introducing third dose mumps vaccine depends in part on the mumps antibodies neutralizing capability after several years from the second dose of measles-mumps-rubella (MMR) vaccination.

#### METHODS

To investigate this issue, we evaluated the mumps neutralizing antibodies present in the serum of three individuals age groups, by plaque reduction neutralization tests (PRNT). First group, 8-9 years old (1-2 years after second dose vaccination); second group, 16-18 years old (10 years after vaccination) and third group, 26-27 years old (20 years after vaccination). PRNT was used to determine the ability of these individuals' serum samples to neutralize the genotype A, Jeryl Lynn mumps virus vaccine strain, and the wild-type virus genotype G5 isolated during the 2014 mumps outbreak in Israel.

#### RESULTS

Unlike, the geometric mean neutralizing antibody titers (GMT) against the Jeryl Lynn strain, which stayed the same in all age groups tested (around 70), the GMT of neutralizing antibodies protecting against genotype G5 declined significantly from 64.5 in the serum samples of the 8-9 years age group, to 41.5 in the 16-18 years age group. Surprisingly, there was no further decline in neutralization antibody titers in the 26-27 years age group.

#### CONCLUSIONS

Our findings show that the PRNT performed against mumps vaccine strains does not reflect the protection against wild type mumps G5 strain. In addition, our results showed that the individual's susceptibility to wild type mumps virus infections is the same after 10 and 20 years even after the second dose of MMR vaccination. In conclusion, our results illustrated that in-depth investigations into waning mumps immunity is needed which might help in reexamining mumps virus vaccination policies.

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### LABORATORY DIAGNOSIS OF GENITAL HERPES INFECTIONS AND STRAIN PROPAGATION - THE IMPACT OF SPECIMENS' MANAGEMENT: RETROSPECTIVE ANALYSIS (2015-2017)

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#### BACKGROUND-AIM

HSV is the main cause of genital ulcer disease but clinical diagnosis is limited in accuracy so laboratory confirmation is recommended. National Reference Laboratory draws attention for the impact of specimens' management for diagnosis, strain propagation and storage and evaluates the diagnostic relevance based on analysis of cases sent to NIH-Lisbon in the last 2 years.

#### METHODS

134 ulcer genital/urethral swabs from patients suspected of herpes and 30 samples of 16 newborns for neonatal HSV diagnosis were sent to NIH between 01/2015-05/2017. HSV 1 and 2 were determined by RT-qPCR and swabs were inoculated in Vero cultures for determination of cytopathic effect and viral propagation.

#### RESULTS

In swabs suspected of genital herpes infection, RT-qPCR and conventional culture are performed; in cultures with cytopathic effect, virus is propagated, quantified and stored at -80°C/liquid nitrogen for further genetic characterization, transmission and antiviral studies.

HSV were detected in 65(48.5%) swabs. 15(23.1%) were HSV1 DNA positive with viral loads (VL) between 1956–87474493 cop/ml, mainly primo-infections and 50(76.7%) were HSV2 with VL between 137–526335906 cop/ml. There was no correlation between VL/primary vs recurrence/subtype. Positives were treated with valacyclovir and resolved. Cytopathic effect and viral propagation was observed in 12(80.0%) HSV1 and in 29(58%) HSV2. HSV1 DNA was detected in CSF/serum/vesicular aspirate of 1(6.3%) case of neonatal herpes diagnosis with viral propagation succeed in the vesicular aspirate.

#### CONCLUSIONS

48.5% of cases were positive for suspected HSV genital infection, with an increasing proportion of HSV1 primo-infection. HSV typing allows earliest appropriate treatment, reducing duration of illness and transmission. DNA detection is the gold standard method for diagnosis, increasing HSV detection rates in swabs. The isolation rate was 80.0% for HSV1 and 58% for HSV2, with lower VL for both types and HSV2 recurrences associated with unviability. Refrigerated quickly transport is mandatory as virus infectivity is heat labile and affects mainly those with low VL. Culture is required for antiviral and transmission studies; correct measures of collection, transport and storage is essential to the success of viral propagation and establishment of strain storage.

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### 25-HYDROXYVITAMIN D3 AS AN IMMUNOMODULATOR IN HERPES SIMPLEX VIRUS -1 INDUCED ANTERIOR UVEITIS IN RABBITS

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#### BACKGROUND-AIM

The activation of pro-inflammatory cytokines via pattern recognition receptors plays an important role in the pathogenesis of HSV-1 uveitis. The role of vitamin D as an immune modulator has recently been studied in various viral infections. This study aimed to evaluate the immunomodulatory properties of vitamin D in a rabbit model of HSV-1 uveitis.

#### METHODS

The study was carried out on 36 rabbits: 18 HSV-1 infected (intracameral inoculation of 105p.f.u. of HSV-1) and 18 mock infected. Out of these, 18 rabbits (9 HSV-1 infected and 9 mock infected) were supplemented with 25-hydroxyvitamin D3 (25D3) for 8 weeks before inoculation and the controls were unsupplemented rabbits. Three rabbits from each group were sacrificed on day 1, day 3 and day 5 post infection (p.i.). The mRNA expression of various TLRs (2, 3, 4, 9), RIG-1, MDA5, NOD2, STING, TNF $\alpha$ , IL (1 $\alpha$ , 4, 6, 8, 10, 12A, 17A, 23A), IFN $\gamma$  and INF $\beta$  were studied and histology was performed. The HSV-1 DNA load was estimated in the aqueous humour. The levels of 25D3 were estimated in serum and aqueous humour using UHPLC-MS/MS.

#### RESULTS

The supplementation of 25D3 resulted in a significant increase in the levels of 25D3 in both serum and in aqueous humour. A decline in clinical signs after HSV-1 infection was observed in 25D3 supplemented rabbits vis a vis unsupplemented rabbits. The expression of TLR2, STING were found to be significantly down regulated (>2 fold, p<0.05) on day 1 HSV-1 p.i. Also RIG-1, MDA5 and NOD2 were significantly downregulated (>2 fold, p<0.05) on day 3 HSV-1 p.i. in supplemented vs unsupplemented groups. A significant decline in HSV-1 load after 25D3 supplementation was observed on day 1 (1229.3 vs 517.5 copies/ $\mu$ l, p<0.05) and day 3 (968.2 vs 497.1, p<0.01) p.i. However, no significant change in the mRNA levels of IFN $\gamma$  and INF $\beta$  were observed after 25D3 supplementation. The proinflammatory cytokines were also found to be down regulated by >2 folds after 25D3 supplementation. The histological examination of eyes showed mild to moderate decline in the inflammation after 25D3 supplementation in HSV-1 inoculated rabbits.

#### CONCLUSIONS

25D3 modulates the inflammatory response after HSV-1 anterior chamber inoculation.

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### SEROPREVALENCE OF EPSTEIN BARR VIRUS

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#### BACKGROUND-AIM

Epstein-Barr Virus (EBV) is a prevalent infection that is present in over fifty percent of eight year old children and an estimated ninety percent of the human population.

In the UK, serology testing is performed pre-donation to establish EBV status and risk of recrudescence. However, a 2015 publication by Public Health England (PHE) has highlighted uncertainty for the optimal algorithm of serological testing. As a result, most hospital laboratories will only screen patients for specific EBV antibodies according to their test algorithm. Due to the complex range of EBV serological profiles a patient can present with, assays have been developed to target specific antibodies to aide diagnosing viral status. As part of the work performed by the Quality Control Reagents Unit (QCRU), over four hundred plasma packs acquired from blood donations have been investigated for the purpose of creating serological reference reagents to aide in the diagnosis of EBV infection and allow diagnostic laboratories to comply with ISO 15189 guidelines.

#### METHODS

Anonymised plasma donations from healthy donors were sourced from the NHS Blood and Transplant (NHSBT) service at Colindale, London, UK, between January and November 2016. These plasma donations were screened for the presence of EBV early antigen (EA), EBV nuclear antigen (EBNA), and both the IgG and IgM for the viral capsid antigen (VCA), using the DiaSorin Liaison XL platform.

#### RESULTS

Results were tabulated according to the EBV serological profile. Table 1 below shows a summary of these results.

Table 1

| EA           | EBNA   | VCA   | IgM   | VCA   | IgG   |
|--------------|--------|-------|-------|-------|-------|
| Negative     | Result | 318   | 86    | 431   | 45    |
| Positive     | Result | 136   | 369   | 24    | 410   |
| Total        |        | 454   | 455   | 455   | 455   |
| Positive (%) |        | 29.96 | 81.10 | 5.27  | 90.11 |
| Negative (%) |        | 70.04 | 18.90 | 94.73 | 9.89  |

#### CONCLUSIONS

The presence of EBNA antibodies is indicative of previous EBV exposure. From the investigations carried out by QCRU, it can be seen that the 83.54% of the average 'healthy' donor in the United Kingdom has a positive anti-EBNA response. This highlights the importance of screening patients for the presence of these antibodies, especially when patients are already immunocompromised or soon to receive blood or plasma transfusions.

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# INTRAVENOUS IMMUNOGLOBULINS FOR PREVENTION OF BK VIRUS INFECTION IN KIDNEY TRANSPLANT RECIPIENTS ACCORDING TO PRE-TRANSPLANT BK VIRUS NEUTRALIZING ANTIBODY TITERS: A PILOT STUDY

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## BACKGROUND-AIM

In kidney transplantation (KT), BKV replication could lead to BKV-associated nephropathy and graft loss. We and others have previously demonstrated that BKV replication post-KT is mostly of donor's origin. Currently, there are no BKV-specific antiviral therapies. In a previous work, we demonstrated that BKV genotype-specific neutralizing antibodies (NAb) are protective against BKV replication above the threshold of 4 log<sub>10</sub> (Solis et al, ESCV 2016) and intravenous immunoglobulins (IVIg) have an important anti-BKV neutralizing activity in vitro and ex vivo (Velay et al, personal communication).

## METHODS

We investigated the efficiency of IVIg for prevention of BKV replication after KT. Patients undergoing KT in Strasbourg were prospectively included from January 1 to March 2, 2017. Donors and recipients NAb were measured the day of KT. Patients at high risk of BKV replication were defined as those having BKV NAb titer below 4 log<sub>10</sub> against the donor's BKV genotype or against the most common genotype (genotype I) if the sample of the donor was unavailable. Patients at risk received 3 cures of IVIg spaced by 3 to 4 weeks at a dose of 0.4 g/kg. The first cure was started at day 21 +/- 7 after KT. BKV NAb titer, viruria and viremia were monitored until 3 months (M3) after KT. Bayesian methods was used to analyze the probability to reach a NAb titer of 4 log<sub>10</sub> against the target genotype after IVIg injection and to estimate the posterior distribution of risk of viruria and viremia at M3 after KT. Data of viruria and viremia from a previous cohort (Solis et al ESCV 2016) were compared to those from the present cohort to determine the impact of IVIg administration on the incidence of viruria and viremia.

## RESULTS

22 patients were included. 12 patients were at high risk for BKV replication and were treated by IVIg. At M3, the NAb titer against the target BKV genotype increased above 4 log<sub>10</sub> (probability: 77%). The risk of viruria and viremia at M3 was decreased in treated patients with a probability of 91% and 81%, respectively.

## CONCLUSIONS

IVIg may represent an important strategy to prevent BKV replication after KT. A larger cohort study with a long-term follow-up are needed to confirm these results.

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# DESIGNING A NOVEL METHOD TO GENERATE RECOMBINANT HERPES SIMPLEX VIRUSES FOR THE CHARACTERIZATION OF UL23 THYMIDINE KINASE MUTATIONS

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## BACKGROUND-AIM

Prolonged use of acyclovir (ACV) in patients undergoing herpes simplex virus type 1 or type 2 (HSV-1/-2) infections may result in the development of drug resistance matching with persisting lesions during ongoing therapy. The prevalence of HSV resistance to ACV is estimated between 2% and 15% among immunocompromised patients. ACV resistance typically results from mutations in the viral UL23 thymidine kinase (TK) gene responsible for the initial phosphorylation of ACV. Molecular diagnosis of HSV resistance to antivirals is challenging since the contribution of numerous mutations remains to be determined. In order to constitute a genotypic database with newly described mutations, mutagenesis of HSV genome included in a bacterial artificial chromosome (BAC) vector was used to generate recombinant viruses containing viral TK sequence variants found in clinical specimens.

## METHODS

The genotypic analysis of HSV-1 or HSV-2 TK gene sequences from clinical samples revealed well-known natural polymorphisms and unpreviously characterized mutations. Mutated TK were transferred into an HSV-2 BAC vector (kind gift from J. Cohen, NIH/NIAID, United States). Recombinant viruses were generated after BAC transfection into Vero cells, and the role of unknown mutations was assessed after evaluation of ACV susceptibility of recombinant viruses by plaque reduction assay (PRA).

## RESULTS

Sensitive (wild-type TK) and resistant (TK with a well-known mutation conferring ACV-resistance) control strains were phenotyped for ACV susceptibility to validate our recombinant phenotyping test. Amino acid changes S66P in HSV-2 TK and G129R in HSV-1 TK were found to confer significant ACV resistance, while mutation A72S in HSV-2 TK was defined as a natural polymorphism since the recombinant virus remained susceptible to ACV using PRA.

## CONCLUSIONS

Recombinant phenotyping has been previously used to characterize antiviral resistance for cytomegalovirus (CMV), varicella-zoster virus (VZV), and HSV-1. To the best of our knowledge, the work presented here reports the first use of BAC technology to assess the contribution of mutations in HSV-2 TK. Our results complete the database of HSV TK mutations with novel natural polymorphism and ACV-resistance mutations.

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**LONG-TERM FOLLOW-UP OF TORQUETENOVIRUS VIREMIA AFTER KIDNEY TRANSPLANTATION**M. Solis<sup>3</sup>, A. Velay<sup>3</sup>, P. Gantner<sup>3</sup>, J. Bausson<sup>2</sup>, A. Filippetti<sup>2</sup>, R. Freitag<sup>2</sup>, B. Moulin<sup>1</sup>, S. Caillard<sup>1</sup>, S. Fafi-Kremer<sup>3</sup><sup>1</sup>Inserm UMR S1109, LabEx Transplantex, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France. Département de Néphrologie - Transplantation, Hôpitaux Universitaires de Strasbourg, Strasbourg, France<sup>2</sup>Laboratoire de Virologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.<sup>3</sup>Laboratoire de Virologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France. Inserm UMR S1109, LabEx Transplantex, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France.**BACKGROUND-AIM**

New biomarkers are needed to accurately assess the degree of immunosuppression in transplant recipients and provide an optimal personalized balance between rejection and infection risks. The main objective of our study is to investigate Torquetenovirus (TTV) loads kinetics at transplantation and for 24 months thereafter in a well-characterized cohort of kidney transplant recipients (KTR).

**METHODS**

Four hundred and twenty blood samples from 70 KTR were collected on the day of transplantation (D0), and at 1, 3, 6, 12 and 24 months (M) after transplantation. Among these KTR, 52 were viruric for BK virus (BKV), of which 28 were BKV viremic including 13 with biopsy-confirmed BKV associated-nephropathy. TTV viremia was measured using the TTV R-gene® kit (bioMérieux, Marcy l'Etoile, France) and analyzed according to patients' characteristics and to their BKV status. Bayesian methods were used to estimate TTV viremia distribution during follow-up using a mixed effects linear model. ROC curve analysis was used to analyze the association of TTV viremia with acute rejection occurrence and BKV status.

**RESULTS**

Positive TTV viremia was detected in 94% of KTR. Mean viral loads were 2.89, 4.23, 6.55, 5.99, 5.14 and 4.53 log<sub>10</sub> copies/mL at D0, M1, M3, M6, M12 and M24, respectively. TTV viremia rose by ≥2 log<sub>10</sub> copies/mL from baseline to M3 (probability of 98%), then declined by ≥1 log<sub>10</sub> copies/mL from M3 to M24 (probability of 81%). Higher TTV viremia was associated with a deceased donor, a lower count of CD8<sup>+</sup> T cells, and a higher BKV viremia (probability of 90%). ROC curve analysis showed that a D0 TTV viremia under 3.3 log<sub>10</sub> copies/mL was associated with acute rejection occurrence after kidney transplantation (AUC 0.747, p= 0.008; sensitivity: 75%; specificity: 62%). An increase of TTV viremia by 1 log<sub>10</sub> copies/mL between D0 and M1 was associated with BKV viruria development (AUC=0.656; p= 0.068; sensitivity: 64%; specificity: 75%).

**CONCLUSIONS**

TTV viremia may adequately reflect the degree of immunosuppression in transplant recipients and represent a useful predictor of acute rejection and BKV replication after kidney transplantation. Future studies on larger cohorts are needed to confirm these results.

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**MANNOSIDE GLYCOLIPID CONJUGATES EFFICIENTLY INHIBIT BK VIRUS INFECTION**W. Kack-Kack<sup>1</sup>, M. Solis<sup>1</sup>, A. Velay<sup>1</sup>, E. Soulier<sup>1</sup>, C.G. Mueller<sup>2</sup>, E. Schaeffer<sup>2</sup>, H. Barth<sup>1</sup>, S. Fafi-Kremer<sup>1</sup><sup>1</sup>Laboratoire de Virologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France ; Inserm UMR S1109, Laboratory of Excellence Transplantex, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France.<sup>2</sup>Laboratory of Immunopathology and Therapeutic Chemistry, CNRS UPR 3572/Laboratory of Excellence MEDALIS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France.**BACKGROUND-AIM**

BK (BKV) virus-associated diseases represent a growing medical issue. To date, there are no BKV-specific antiviral therapies available. Newly synthesized mannoside glycolipid conjugates (MGCs) formed by three building blocks comprising a trimannose head, a hydrophilic linker and a hydrophobic lipid chain, have been described as antiviral agents interacting with the cell membrane (Dehuyser et al, Bioconjug Chem. 2012). We aimed to investigate the potential efficacy of these compounds to inhibit BKV cell infection.

**METHODS**

Anti-BKV activity of 13 MGC molecules was investigated using a BKV pseudovirion (PsV) system expressing the capsid proteins of BKV genotypes I, II, III or IV, and using the entire virion BKV Dunlop. The inhibition concentration was defined as the molecule dilution that yielded 50% inhibition (IC<sub>50</sub>) of PsV or of the entire virion infection.

**RESULTS**

Two (TriManC24 and TriManC24U) out of 13 molecules efficiently inhibited BKV PsV infection, 7 showed no or low activity and 4 induced cell toxicity. The mean IC<sub>50</sub> of TriManC24 was 12.94 μM, 11.7 μM, 15.43 μM for genotype I, II and III, respectively. Those for TriManC24U were 47.48 μM, 46.89 μM and 54.57 μM for genotype I, II and III, respectively. The antiviral activity of the two molecules was significantly lower for genotype IV (p<0.005) with a mean IC<sub>50</sub> value of 38.26 μM for TriManC24 and >100 μM for TriManC24U. The antiviral activity of the 2 molecules was confirmed on the entire virion system (BKV Dunlop). Structure comparison of the 13 molecules demonstrated that the presence of a saturated lipid chain is crucial for antiviral activity, while sugar moiety modification leads to cell cytotoxicity.

**CONCLUSIONS**

The MGCs TriManC24 and TriManC24U efficiently inhibit viral infection of BKV genotype I, II and III and in a lesser manner of genotype IV. This suggests that MGCs may interfere with BKV cell receptors - gangliosides containing sialic acid motifs - required for the cell infection of genotype I, II and III but not necessarily for genotype IV. Further analyses are needed to elucidate the mechanism of action of these two compounds.

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# **SIMPLE, FAST AND REPRODUCIBLE PHENOTYPIC RESISTANCE DETERMINATION FOR HERPES SIMPLEX VIRUS BY FLOW CYTOMETRY.**

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## **BACKGROUND-AIM**

Prolonged use of acyclic nucleoside analogues (acyclovir, penciclovir) or a pyrophosphate analogue (foscarnet) can result in drug resistance. The goal of this study was to develop a simple, fast and reproducible method for resistance determination by flow cytometry.

## **METHODS**

Twenty-five previously characterized HSV-1 isolates from 14 patients were used(1). These isolates were tested for resistance by UL23, 30 sequence analysis, plaque reduction assay (PRA) and DNA reduction assay (DRA). A susceptible and resistant isolate was used for testing the procedure with different multiplicities of infection (MOI). Furthermore we used 3 different fresh isolates to determine throughput time in clinical practice. Reproducibility tests were performed by two different technicians. Verocells were grown in 25 cm<sup>2</sup> flasks. Virus was added together with either acyclovir or foscarnet in a twofold dilution series in culture medium. After 16-24 hrs incubation medium was removed. Cells were released incubated with HSV-1 glycoprotein (Diagnostic Hybrids; HSV-1 DFA reagent) (no permeabilisation) and measured in a Beckman Coulter FC500-MCL at 488nm wave length. Percentage of gated infected (fluorescent) cells were used. Susceptibility to antiviral drugs was determined by calculation of the concentration of drug (micromolar) that reduced infected cells by 50% (IC<sub>50</sub>).

1. Van der Beek et al. Rapid susceptibility testing for herpes simplex type 1 using real-time PCR. JCV 2013 vol 56 pp 19-24

## **RESULTS**

Full agreement was obtained between the different methods. The test could be performed within 16-24 hrs. The results were similar in a wide range of multiplicities of infection and reproducibility was high.

## **CONCLUSIONS**

We wanted to evaluate different phenotypic techniques for susceptibility determination for HSV with culture. The goal was a simple, reproducible and fast method. The FACS with labeled antibodies against HSV proves to be fast and reproducible without having to permeabilize the cells. The antibodies binding to the celmembrane of infected cells were sufficient to early recognize infected cells. One drawback was that you need enough cells for measurement. We had to work with 25 cm flasks we are in process of testing 6 well plates.

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# **INTERFERON INDUCIBLE PROTEINS AS BIOMARKERS OF RESPIRATORY VIRUS INFECTIONS**

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## **BACKGROUND-AIM**

Expression of interferon stimulated genes and corresponding proteins has been shown to be associated with respiratory virus infections, yet their potential as inflammatory biomarkers have not been clinically exploited. We have shown earlier that in young (<2 years of age) children, elevated MxA levels were strongly associated with symptomatic respiratory virus infections, including those caused by rhinoviruses. MxA levels in children who had recently received a live virus vaccination were significantly higher than in controls, but lower than in those with symptomatic infection.

## **METHODS**

We assessed myxovirus resistance protein A (MxA) in PCR-confirmed respiratory virus infections by using a laboratory developed enzyme immunoassay (EIA), that has been validated for the measurement of MxA levels in lysed whole blood samples from MS patients treated with interferon. A commercial EIA was used to measure serum levels of tumor necrosis factor-related apoptosis inducing ligand (TRAIL), also expressed by interferon stimulation. RT-PCR was used to measure mRNA expression levels for interferon inducible genes MxA, viperin, and tripartite motif-containing protein 21 (TRIM21).

## **RESULTS**

MxA and viperin, but not TRIM21 mRNA levels in nasal swab specimens correlated with MxA protein levels in blood. As compared to MxA, the mRNA levels of viperin provided even better differentiation between symptomatic and asymptomatic infections. In 1–16 years old children with acute pharyngitis, blood MxA levels were elevated in infections involving viruses but not in those with group A streptococcus as the sole pathogen. In addition, MxA had stronger association with virus infection than TRAIL.

## **CONCLUSIONS**

Measures of expression of interferon stimulated genes have great potential as biomarkers of respiratory virus infections – as a surrogate or an extension of pathogen specific diagnostic methods.

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# INTERIM ANALYSIS OF A CLINICAL TRIAL OF A NOVEL IMMUNOLOGICAL ASSAY, QUANTIFERON-MONITOR®, IN THE MANAGEMENT OF INFECTIONS IN HEART TRANSPLANT RECIPIENTS (HTR)

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## BACKGROUND-AIM

Infections are a major complication after solid organ transplant. An interim analysis of a clinical trial of a novel immunological assay, the QuantiFERON-Monitor® (QFM®; Qiagen-USA), in identifying heart transplant recipients (HTR) at higher risk of developing infection is reported.

## METHODS

QFM® assay measures IFN- $\gamma$  levels (IU/mL) in plasma after incubation of 1 mL of heparinized whole blood with innate (R848) and adaptive (CD3) stimulants. The assay was performed as per protocol's instructions at study entry and 3 months later. The occurrence of symptomatic and/or requiring treatment infection within 6 months from the enrollment was the study endpoint.

## RESULTS

Among the 131 HTR enrolled (time after HT: <6 months; 6-5 years; >5 years), 51 (38.9%) developed at least an infectious episode (total number=69). Significantly lower median IFN- $\gamma$  levels in patients at <6 months from transplant were detected [18 [25-75th percentile, 11-148] vs. 181 [62-523] IU/mL;  $P<0.0001$ ] as well as in patients who developed a post-transplant infection [66 [14-177] vs. 186 [57-650] IU/mL, respectively;  $P<0.0001$ ]. Moreover, significantly lower median IFN- $\gamma$  levels in patients who developed viral infections [90 [20-152] IU/mL] compared to infection-free patients were observed ( $P=0.0009$ ). Viral infections ( $n=39$ ) were involved in 56.5% of cases. Cytomegalovirus ( $n=27$ ), herpes simplex virus ( $n=1$ ), varicella zoster virus ( $n=1$ ) and influenza virus ( $n=3$ ) were detected; in 7 cases diagnosis of infection was made on clinical signs. Patients who developed bacterial infections also had lower median IFN- $\gamma$  levels [42 [13-213] IU/mL] than infection-free patients ( $P=0.0003$ ). Finally, patients with recovering infection showed higher median IFN- $\gamma$  levels than those with relapsing or de novo infections ( $P=0.0004$  and  $P=0.0020$ , respectively). Sufficient data are not available to identify a reliable QFM® threshold predicting the infectious risk.

## CONCLUSIONS

These preliminary results suggest that QFM® measurements may be a support tool to identify HTR at higher risk of infection.

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# INHERITED CHROMOSOMALLY INTEGRATED HHV-6 (ICIHHV-6): DIAGNOSIS OF THE PRESENCE OF ICIHHV-6 AND ESTIMATION OF THE NUMBER OF HHV-6 GENOMIC COPIES INTEGRATED USING QUANTITATIVE REAL-TIME PCR AND DROPLET DIGITAL PCR ASSAYS

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## BACKGROUND-AIM

In 1% of the population, integration of HHV-6 genome in the telomeric region of one chromosome in each cell, including hair follicles and nails, was evidenced. According to laboratories, number of integrated copies differs from 1 to 7/cell.

The aim of this study was (i) to determine the number of HHV-6 genomic copies integrated using real-time and droplet digital PCRs, (ii) to compare the number of integrated genomes for HHV-6A and HHV-6B, (iii) to analyze viral load in different matrices for a same patient.

## METHODS

Eighty one samples from 50 patients with iciHHV-6A ( $n=20$ ), iciHHV-6B ( $n=29$ ), and one unidentified, were studied. Samples were 50 whole blood, 16 hair follicle, 8 nail, 5 cerebrospinal fluid (CSF), 1 bronchoalveolar fluid, and 1 colonic biopsy. Twenty five patients had from 2 to 4 samples. Extraction of DNA (viral and cellular) was performed using different methods according to the period and matrix (MagnaPure, Qiasymphony, EasyMag, QIAamp DNA Blood MiniKit, QIAamp DNA MiniKit).

HHV-6 viral load (VL) was measured using (i) RT-PCR method targeting U65-66 genes previously developed (Gautheret-Dejean et al. J Virol Methods 2002), (ii) ddPCR for U42 developed by P. Bonnafous. For both methods, the number of HHV-6 genomes per cell was determined by a parallel quantification of the gene coding for albumin (Laurendeau et al. Clin Chem 1999).

## RESULTS

HHV-6 VL differed according to the method used. Considering all samples, median VL was 4.7 copies/cell [1.1-329] using RT-PCR, and 1.0 copy/cell [0.1-50] using ddPCR. No correlation could be evidenced between both methods ( $p=0.85$ ;  $R^2=0.001$ ). When separating iciHHV-6A and iciHHV-6B, both

methods did not correlate ( $p=0.8$  for iciHHV-6A,  $p=0.27$  for iciHHV-6B). When matrices were analyzed separately, median VLs were similar for ddPCR in whole blood (1), hair follicles (1.1), nails (1), but lower in CSF (0.6). Analogous but higher results were observed for RT-PCR in whole blood (4.3), hair follicles (4.9), nails (5.2), but not in CSF (0.6).

#### CONCLUSIONS

In iciHHV-6 patients, HHV-6 genomic median copy number was estimated at 1 copy/cell using ddPCR, and 4 copies/cell using U65-66 RT-PCR. This number was identical in whole blood, hair follicles, nails. Further studies are needed to understand these differences.

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#### ENSURING THE QUALITY OF BK POLYOMAVIRUS QUANTITATIVE DETECTION USING THE 1ST BKV WHO INTERNATIONAL STANDARD

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#### BACKGROUND-AIM

The reactivation of BK polyomavirus poses a significant risk in immunocompromised kidney transplant recipients, raising the incidence of renal dysfunction and graft loss caused by BKV associated nephropathy (BKVAN). Guidelines for the management of these patients recommend periodic screening of BK viral load post-transplantation, to guide immunosuppressive prophylaxis. However the implementation of a universal viral load treatment threshold of 4.0 log<sub>10</sub> copies/ ml cannot be reliably achieved across institutions without effective standardization of diagnostic NAT assays.

#### METHODS

We worked with the WHO's Expert Committee for Biological Standardisation to establish the 1st WHO International Standard for use as a primary order calibrant for BKV NAT assays. However subsequent characterisation of the material using NGS revealed the presence of truncated sub-populations. This was reported in a recent publication that questioned the suitability of the WHO BKV IS as a primary calibrant.

#### RESULTS

We have performed NGS characterisation of the WHO BKV IS using short- and long- sequencing technologies and independently confirmed the presence of truncated genomes. Nevertheless our data from the qPCR evaluations performed in the multi-centre collaborative study demonstrate there is no significant difference in viral load quantification across the various genomic targets. Using relative potency with the BKV IS, we show harmonisation of data across the participating laboratories that closely resemble the reduction in variability obtained with a second candidate material comprised only of full-length sequence. By contrast similar harmonisation was not achieved when a synthetic plasmid was used as a calibrant. Further analyses are ongoing to ensure the suitability of the WHO IS for ddPCR assays.

#### CONCLUSIONS

Our findings endorse the use of the BKV WHO IS as a primary calibrant that is able to effectively quantify BK viral load using qPCR. Its use should support the implementation of a more consistent clinical management approach to BKV reactivation, through the adoption of the universal viral load treatment threshold.

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### NEW FRET-BASED ANTIBODY ASSAY FOR ASSESSMENT OF THE IMMUNITY STATUS AND LIFELOG INFECTION HISTORY

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#### BACKGROUND-AIM

In today's laboratory medicine the emerging megatrend is point of care (POC) diagnostics. This requires rapid and robust assays that can be carried out without intensive labor or extensive machinery.

We have previously developed a diagnostic concept based on fluorescence resonance energy transfer (FRET), for on-site rapid measurement of specific antibodies. Briefly, FRET between two fluorophores, donor and acceptor, is elicited if they are in close proximity (<10 nm). An interaction between an antigen and the specific antibody will bring together the acceptor and the donor coupled to these molecules, resulting in FRET fluorescence at the specific wavelength. This new serodiagnostic concept is homogenous (wash free), and yields results rapidly after combining the reagents. Our research partners at Technical Research Centre of Finland have developed a portable, battery operated prototype fluorometer for field use of our FRET antibody assays.

This has been successfully (sensitivity 100-95 % and specificity 100-99 %) applied to the diagnosis of acute hantavirus disease (IgM), but the performance in detecting past immunity or latency (IgG) has been poor.

#### METHODS

We now present a competitive, homogenous FRET-based antibody assay suitable for assessment of the immunity status and life-log infection history. The Pilot assay was developed for parvovirus B19, using B19V IgG and IgM EIAs as reference.

The B19V immunity status was assessed with 186 well-characterized IgG-positive and IgM-negative sera from tonsillectomy patients. The performance in IgM detection was investigated with 18 IgG-negative and IgM-positive sera from individuals with acute B19V infection.

#### RESULTS

The clinical sensitivity of the new assay in detection of B19V IgG was 99 % and the specificity was 97 %. The assay did not detect IgM antibodies, as indicated by sensitivity of 22 %.

#### CONCLUSIONS

The new assay is well suited for IgG detection. Homogenous assay format, e. g. direct measurement after combining the reagents, allows further translation into POC diagnostics.

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### ENHANCED SURVEILLANCE OF NON-POLIO ENTEROVIRUSES AND THE MANIFESTATION OF ENTEROVIRUS D68 IN EUROPE, 2016

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#### BACKGROUND-AIM

In the context of what we call 'diagnostic stewardship', Clinical laboratories in the Northern part of the Netherlands have set up a real time sequencing network for surveillance of enterovirus (EV), norovirus and parechovirus. This initiative – REGIOTYPE – is aimed to provide clinical-, phylogenetic- and sequencing data on circulating viruses to gain insight into local epidemiology as well as transmission networks within and between healthcare institutions.

Like in 2009/2010 and 2014, an upsurge of EV-D68 infections was recorded during the summer of 2016. More than 25 respiratory cases were observed with many Intensive Care admissions as well as one case of Acute Flaccid Myelitis (AFM).

#### METHODS

Observations from our region were rapidly shared (inter) nationally and a questionnaire on the presence of EV-D68 and/or EV-D68 associated AFM cases was submitted to European laboratories within the EV-D68 working group.

#### RESULTS

Many EV-D68 respiratory infections were detected throughout 22 European countries. At least 32 cases of EV-D68 related to AFM were observed, an almost 10-fold increase compared to 2014. Information needed to estimate the burden of disease around neighboring regions and countries was broadly available but simply not shared across different specialisms, institutions and borders. Sequence information identified a new subclade B3 of EV-D68 circulating in 2016, which was not previously found in our region.

#### CONCLUSIONS

Sharing sequencing data, combined with epidemiological data and clinical information that is primarily provided by local level initiatives like REGIOTYPE, contributes significantly to the knowledge of circulating EV(-D68) infections and AFM cases throughout Europe. This should also contribute to increased awareness among clinicians (e.g. neurologists or infectious disease specialists). However, some regions in Europe are still underreporting or not-reporting the aforementioned information.

Forming clinical networks and providing diagnostic support are key fundamentals in a (currently lacking) real-time non-polio enterovirus (NPEV) surveillance system, for the benefit of the patients. Communication within these networks is essential.

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# A CASE REPORT DEMONSTRATING THE UTILITY OF NEXT GENERATION SEQUENCING FOR DETECTION OF ANTIVIRAL RESISTANCE MUTATIONS FROM A TRANSPLANTED PATIENT WITH VZV INFECTION

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## BACKGROUND-AIM

Heart transplantation (HT) is associated with the risk of reactivation of varicella zoster virus (VZV). Valacyclovir (VACV) prophylaxis is recommended after HT in seropositive recipients. Antiviral prophylaxis may fail when drug resistance mutations (DRMs) are selected. This retrospective study aimed at showing the added value of ultra-deep sequencing (UDS) in a case of resistant VZV infection initially detected by Sanger sequencing.

A 47-year-old man, seropositive for VZV, underwent HT consecutive to cardiac amyloidosis on April 27, 2016 after receiving one year of VACV prophylaxis. After HT, he rapidly presented post-transplant graft dysfunction and kidney failure requiring extracorporeal membrane oxygenation. After several episodes of ventilator-associated pneumonia and pulmonary aspergillosis treated with antibiotics and antifungals, he developed herpes zoster on May 30, 2016 related to intensive immunosuppressive treatment for the prevention of graft rejection (i.e., antithymocyte globulin, mycophenolate mofetil, corticosteroids, and tacrolimus). Virological response was complete under low-dose of VACV adapted to renal function. On July 7, 2016, zoster lesions reappeared and intravenous ACV was started. As VZV resistance mutation was evidenced on July 21, 2016, ACV was switched to foscarnet with significant improvement.

## METHODS

Viral DNA was extracted from serial VZV-positive clinical samples recovered during treatment failure. Full-length ORF36 (encoding thymidine kinase, TK) and ORF28 (encoding) DNA polymerase were sequenced by Sanger method and UDS after shotgun strategy using the MiSeq® platform (Illumina).

## RESULTS

Within TK, major DRMs (>20%) were identified both by Sanger and UDS methods: thymidine insertion generating a frameshift and Q69Stop mutation; whereas minor variants (<20%) were detected by UDS only: two different cytidine deletions generating frameshifts. Interestingly, DRMs could be simultaneously detected in some samples. No resistance mutations were detected in DNA polymerase.

## CONCLUSIONS

This case report revealed a complex dynamics of VZV resistance under antiviral pressure. The emergence of successive DRMs could be roughly detected using Sanger method whereas UDS allowed better characterization of the viral population by identifying additional DRMs.

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# MONITORING LIVE VACCINE UPTAKE IN CASE OF TWO AVIAN HERPESVIRUSES

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## BACKGROUND-AIM

Protection against diseases caused by two avian herpesviruses, Marek's disease (MDV) and Infectious laryngotracheitis (ILT) is achieved by live vaccines. The vaccine application process quality is important to assure proper uptake in commercial flocks.

## METHODS

We suggest evaluating the vaccination process by monitoring the presence of the live vaccine viruses in the bird feathers.

## RESULTS

Specific identification of the vaccine virus by a newly developed DIVA assay ensured the detection of the vaccine virus and not the wild-type viruses. We present the use of feathers as a non-invasive organ for examination, because feathers are easy to collect, non-lethal for the bird, therefore advantageous for monitoring purposes. Surveying vaccine viruses indicated the vaccine application quality, and correlated to the vaccine doses and various vaccination routes.

## CONCLUSIONS

By the novel approach and methodology it was demonstrated that the vaccine virus reside in vivo for at least 94 days post vaccination, the time period that was monitored. However, the vaccine might be detected further due to the herpesvirus persistency. The monitoring method suggested is indicative of the vaccine application quality. Although the study employed avian herpesviruses, the principles presented can be applied for human and animal viral vaccine viruses.

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### ANTIVIRAL ACTIVITY OF AQUEOUS FULLERENE C60 DISPERSION IN THE TREATMENT OF CUTANEOUS HSV1 INFECTION IN MICE

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#### BACKGROUND-AIM

Herpes simplex viruses (HSV1 and HSV2) are widely spread in human population, being a potential cause of serious diseases (often recurrent) in individuals with immunodeficiency. Chemical therapeutic agents used in these cases have high toxicity and induce drug resistance. Low-toxic water-soluble fullerene C60 derivatives display antioxidant and membrane tropic activities in vitro. However, anti-HSV therapeutic activity of fullerenes has not been studied in sufficient detail.

The aim of this work was to evaluate anti-HSV1 activity of aqueous dispersion fullerene C60 and three water-soluble C60 derivatives in vitro and in vivo.

#### METHODS

In vitro antiviral activity of aqueous fullerene C60 dispersion (dnC60) and fullerene C60 conjugated to piperazine (C60-Pip), arginine (C60-Arg) and lysine (C60-Lys) was studied in Vero cells. In vivo therapeutic activity was assessed in a mouse model of epidermal cutaneous HSV1 infection. 4 groups each consisting of 5 DBA mice were used. Test agents were applied locally 24 h after infection: dnC60 (10 lg), C60-Pip (50 lg) and 5% acyclovir cream (5000 lg ACV) once a day for 3 days. Saline solution was applied in the control group. The animals were examined daily for 10 days and the development of skin lesions were scored from 0 to 5.

#### RESULTS

C60-Arg and C60-Lys exhibited no antiviral activity in vitro, while dnC60 and C60-Pip suppressed HSV1 infection (ID50 9.4 lg/ml and 13.5 lg/ml, respectively); they were further investigated in vivo. The severity of cutaneous HSV1 infection in the control group was estimated as 3.1±0.4 score, 2 out of 5 mice are died. All animals in experimental groups survived during the observation period. Statistically significant reduction of HSV1 skin lesion was observed in mice treated with dnC60 and ACV: 1.4±0.4 and 0.8±0.5 score, respectively, P<0.05.

#### CONCLUSIONS

Thus, aqueous dispersion of fullerene C60 shows higher anti-HSV1 activity than fullerene C60 amino acid derivatives studied, and its therapeutic effect being comparable with that of ACV applied in a 500-fold higher concentration.

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### COMPARATIVE EVALUATION OF ILLUMIGENE® CMV ASSAY AND REAL TIME COMMERCIAL KIT FOR THE NEONATAL SCREENING OF CONGENITAL CMV INFECTION IN SALIVA SAMPLES

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#### BACKGROUND-AIM

Congenital cytomegalovirus (cCMV) infection is the leading non-genetic cause of sensorineural hearing loss and neurodevelopmental sequelae. Most infants at risk for CMV-associated hearing loss are not identified early in life. Thus the availability, of rapid and reliable diagnostic method that can be adapted for high-throughput screening, is very helpful in order to identify the CMV-infected infants and start their follow-up.

#### METHODS

We evaluated a new molecular assay, illumigene® CMV, for the screening of cCMV infection in saliva samples, compared with a commercial extraction (easyMAGSystem, bioMerieux, France) and real time-PCR (ELITechGroup Molecular Diagnostics, Italy).

The illumigene® CMV (Meridian Bioscience Inc., OH, USA) is a rapid method that provides results within 40 minutes by sample treatment using lysis buffer to release nucleic acid and amplification with LAMP technology. Two workflows were designed to extract DNA from saliva samples, CMV simple filtration SMP-Prep and and M-prep™ device.

#### RESULTS

Fifty-eight saliva specimens, analyzed with illumigene®, were collected with saliva dried swabs (FLOQSwabs, Copan, Italy) from 26 children who were between the ages of 1 day and 48 months (median 4.5 months). All 26 newborns had cCMV infection: 19 were asymptomatic and no antiviral treatment was necessary, 7 were symptomatic. Of these, two newborns had mild and fluctuating hearing-loss and did not require treatment, 5 infants had severe symptoms and underwent valganciclovir treatment. For 17/26 infants, maternal data was available: 15 mothers had primary and 2 non-primary CMV infection during pregnancy. Finally, among the 12 CMV-negative samples, 8 were collected when the infant underwent therapy with valganciclovir; four samples belong to children who were 4 years old.

Only with the M-prep™ workflow we obtained a 100% of sensibility and 100% specificity compared with the reference assay (traditional extraction and real-time PCR). The agreement resulted 100%.

#### CONCLUSIONS

These preliminary and promising findings prompted us to increase the number of samples for further evaluation of illumigene® CMV as an easy and fast molecular assay suitable for the programs of screening of congenital CMV infection.

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# THE FUTURE OF HIGH QUALITY CALIBRATORS FOR DIAGNOSTIC NAT ASSAYS - LESSONS LEARNT FROM 20 YEARS OF PRODUCING INTERNATIONAL STANDARDS ESTABLISHED BY THE WHO ECBS

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## BACKGROUND-AIM

The correct use of biological reference materials are essential to facilitate consistent and comparable data generated by diagnostic assays. Without such, it is impossible to compare results between laboratories or assays. This is problematic when universal treatment thresholds are established. Furthermore, whilst there may be a variety of materials available there is a lack of clarity among users as to their correct use.

For over 20 years, NIBSC, on behalf of the WHO, have been producing international standards. These are the highest order biological reference materials, otherwise known as international calibrators. Highly characterized and assessed in international studies by a range of laboratories using different assays, these materials are assigned an arbitrary unit of value, the international unit.

## METHODS

Like any international calibrator, these materials should be preserved and used only for the calibration of other material. In a survey conducted by NIBSC only 14% of laboratories reported using these materials for this purpose. Moreover, where the material has been used as a calibrator, literature highlights inter assay differences between the resultant calibrated materials, suggesting differences in methodologies. This raises the question among many, how should the international standard be used and what's the difference between types of reference material?

## RESULTS

Drawing on experience, this paper will describe essential steps in the production and calibration of reference materials. It will outline important considerations when selecting suitable analyte types and the importance of specificity and steps to assess stability. Not all material performs equally, the wrong material can de standardise, as experienced when using a CMV plasmid, a >3Log10IU variation was observed compared to < 1.5 Log10 IU where whole virus was used. Protocol designs to provide sufficient data for analysis are considered, whether for a single laboratory or multi centre. Then, once faced with a data set, what statistical analysis is required to assign a calibrated value?

## CONCLUSIONS

Finally, comparable data requires engagement of all manufacturers without which their assays have been seen to over or under quantitate by up to 1 Log10. A good standard is only part of the journey to harmonised results!

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# EVALUATION OF CELLULAR LOAD IN RESPIRATORY SAMPLES FOR THE NORMALIZATION OF VIRAL LOAD.

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## BACKGROUND-AIM

Respiratory tract infections (RTI) have an enormous social economic impact, with high incidence of hospitalization and high costs. Adequate specimen collection is the first crucial step for the correct diagnosis of influenza and other respiratory infections. The present study aimed: i) to verify the cell yield obtained from sampling the nose using the flocked swab and collected in the Universal Transport Medium™; ii) to evaluate the normalization of viral load based on cell numbers.

## METHODS

The number of respiratory epithelial cells were counted by quantifying the DNA of housekeeping gene (beta2-microglobulin) by real-time PCR in residual extract of nasal swabs stored in freezer at -80°C and collected at the Molecular Virology Unit of Fondazione IRCCS Policlinico San Matteo. The results were reported as 2-microglobuline DNA copy numbers/ml of UTM. The normalized value of the viral RNA load was expressed as the number of viral RNA copies/median number of cells recovered.

## RESULTS

A total 739 samples were analyzed in this study. Of them, 513/739 (69.4%) were positive for at least one respiratory virus, while 226/739 (30.6%) were negative. Overall, a median of 4.42 log10 2-microglobuline DNA copy number/ml of UTM was detected and the number of cells yielded by flocked nasal swabs ranged from log10 1.17 to 7.26. A significant high level of cells was observed in virus-positive as compared to virus-negative samples (4.75 vs 3.76; p<0.001). The viral loads expressed as log10 RNA copies/ml of UTM and log10 RNA copies/median number of cells (log10 4.42=45282 cells) were compared in the 513 virus-positive samples. Good correlation (r=0.89, p< 0.001) and agreement (R2=0.82) was observed between the two ways of expressing viral load. The kinetic of infection was evaluated with the two ways of expressing viral load in eight follow-up sample series (range 3-5). A total of 32 samples were analyzed and the mean of difference was -0.57 log10 (median -0.55, range -1.99 to 0.40). Similar viral load dynamics were observed for all the analyzed cases.

## CONCLUSIONS

The normalization of viral load using the cellular load seems to be helpful for the validation of real-time PCR results in the diagnosis of respiratory viruses.

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### COMMUTABILITY AND AGREEMENT OF INTERNATIONAL AND SECONDARY STANDARDS

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#### BACKGROUND-AIM

Quantitative molecular diagnostics is an integral part of health care for both immunocompetent and immunocompromised patients, but commutability of results is problematic due to differences in methodology, chemistry and equipment. The WHO international standards and subsequent secondary standards have been developed for several viruses, aiming to improve commutability of quantitative values between laboratories. However, secondary standards from differing suppliers must be commutable with each other, or they will only be another inter-lab variable. This study aims to investigate the commutability and agreement of primary and secondary international standards, as well as to investigate the option of creating a target-independent universal standard.

#### METHODS

WHO international standards and commercially available secondary standards were compared using three PCR techniques; an LDT on the ABI7500 platform, the BioRad QX200 digital PCR and the EliTech InGenius system. The chosen targets are Cytomegalovirus, Epstein-Barr virus, BK virus, Varicella Zoster Virus, and Herpes Simplex virus type 1 and 2. Secondary standards were obtained from Acrometrix, Exact Diagnostics, NIST, Onostics, Seracare and Zeptomatrix.

#### RESULTS

Differences between the EliTech InGenius and the other two PCR methods were found, while the ddPCR and RT-PCR showed a high level of agreement. However, the agreement between commercial standards was low, with clear differences observed between various suppliers of the same target using the same PCR method. Agreement improved when a WHO international standard was available, but differences could still be as high as 10-fold.

#### CONCLUSIONS

This study shows that agreement between secondary standards is low, implicating that the standard selected by a laboratory will significantly affect their quantitative results. The current secondary standards are currently only another variable when trying to compare results between laboratories. In the absence of international standards for a large number of (viral) targets -added to the lack of agreement between existing standards-, new strategies must be developed to improve commutability of results. We theorise that a target-independent universal standard can be implemented for calibration purposes.

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### A RAPID GENOTYPING ASSAY TO DISCRIMINATE BETWEEN RHINOVIRUS A, B AND C

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#### BACKGROUND-AIM

Human rhinoviruses (RVs) are increasingly associated with severe disease of the respiratory tract. Multiple studies highlighted the clinical significance of different RV species; RV-C is linked to asthma exacerbations and increased disease severity in children, whereas RV-B seems to correlate with milder disease. Current typing strategies for differentiation of RV species are time consuming and require extensive equipment. We present a novel genotyping tool to discriminate between RV species A, B and C.

#### METHODS

The Chipron LCD RV array encompasses a VP4/VP2 polymerase chain reaction (PCR), followed by hybridization of the product on a macro array with probes covering RV-A, B, and C. Validation was performed with respiratory specimens submitted for diagnostic evaluation to the Academic Medical Center. A selection of RV PCR positive samples genotyped by VP4/VP2 was evaluated. Diagnostic performance was tested on respiratory samples positive for RV in an in-house multiplex respiratory PCR from Jan 2016 to Jan 2017. In-house primers and additional isolate-specific primers were used for partial sequencing to investigate array-negative and -double-positive samples.

#### RESULTS

The majority of samples with known RV genotype (n = 135) were classified into the correct species, except for one that was assigned RV-C instead of RV-A, and 3 samples that tested negative. The array gave a double-positive result in 4 cases; the presence of more than 1 genotype was confirmed in 2 samples. In 173/187 (92.5%) RV positive patient samples from 2016, the test resulted in a designated species. RV species A was identified in 109 specimens (58.3%), RV-B in 26 (13.9%), and RV-C in 56 (29.9%) samples. Sequencing of the probe region of 14 (7.6%) negative samples revealed up to 3 mismatches for 12 samples; in 2 cases no PCR product was generated. Notably, the chip detected more than one species in 18 samples, of which 15 were confirmed by sequencing with probe-region-based primers.

#### CONCLUSIONS

The Chipron LCD RV array provides a fast and highly sensitive method for discrimination between rhinovirus species, including detection of dual infections.

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### EVALUATION OF ANALYTICAL SENSITIVITY OF 11 HIV RAPID DIAGNOSTIC TESTS DURING HIV-1 PRIMARY INFECTION

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#### BACKGROUND-AIM

Around the world, a majority of HIV infections is diagnosed using rapid diagnostic tests (RDTs). Their performance in terms of sensitivity and specificity are crucial.

The aim of this study was to evaluate the analytical sensitivity of 11 HIV RDTs on serum samples from patients at the early stage of HIV-1 infection (primary infection).

#### METHODS

74 serum and 1 plasma samples from patients during the primary stage of HIV-1 infection were tested with HIV Combo (Alere), INSTI TM HIV-1/HIV-2 Antibody Test, MULTISURE HIV Rapid Test, SURECHECK® HIV1/2 and HIV1/2 STAT-PAK® Assay (AAZ, Nephrotek), Exacto® TEST HIVPRO et HIVTOP® Ac1&2 (Biosynex), Genie TM Fast HIV1/2 (BioRad), VIKIA® HIV1/2 (BioMérieux). Among them, 73 serum and 1 plasma samples were tested with FIRST RESPONSE® Test VIH1-2.O CARTE (Nephrotek), and 69 serum and 1 plasma samples were tested with Hexagon HIV1&2 (Servibio). For HIVTOP® Ac1&2 and MULTISURE HIV Rapid Test, visual and automated readings were done.

#### RESULTS

RDTs's sensitivity ranged from 73% to 100% with HIV Combo (100%), Exacto® TEST HIVPRO (92%), HIVTOP® Ac1&2 automated reading (91%) and visual reading (89%), Hexagon HIV1&2 (89%), Genie TM Fast HIV1/2 (88%), VIKIA® HIV1/2 (81%), FIRST RESPONSE® Test VIH1-2.O CARTE (77%), INSTI TM HIV-1/HIV-2 Antibody Test, HIV1/2 STAT-PAK® Assay, SURE CHECK® HIV1/2 and MULTISURE HIV Rapid Test visual reading (76%) and MULTISURE HIV Rapid Test automated reading (73%). Invalid results were observed with VIKIA® HIV1/2 (3%), HIVTOP® Ac1&2 visual reading (1.3%) and Genie TM Fast HIV1/2 (1.3%). For MULTISURE HIV Rapid Test, HIVTOP® Ac1&2, Hexagon HIV1&2 and FIRST RESPONSE® Test VIH1-2.O CARTE that allow HIV-1/HIV-2 differentiation, cross-reactivities were observed between 5% and 49%.

#### CONCLUSIONS

Sensitivity of RDTs tested for serum samples during HIV-1 primary infection differed ranging from 73% to 100%. HIV Combo, Exacto® TEST HIV PRO, HIVTOP® Ac1&2, Hexagon HIV1&2 and GenieTM Fast HIV1/2 were the more sensitive. Except FIRST RESPONSE® and MULTISURE HIV Rapid Test with an automated reading, RDTs that allow the differentiation between HIV-1 and HIV-2 exhibited a relatively high level of cross-reactivities. These results may help the users of countries working only with RDTs to choose an optimal combination, as recommended by the WHO.

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### Q-LAMP ASSAY FOR DETECTION OF POLYOMAVIRUS BK

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#### BACKGROUND-AIM

Diagnosis of BKV infection is based on molecular assays such as RealTime-PCR (RT-PCR) with high specificity and sensibility. The DiaSorin Q-LAMP (loop mediated Isothermal amplification- DiaSorin SpA, Saluggia, Italy) offers all the benefits of isothermal LAMP technology with the addition of a real time fluorescent detection and multiplexed amplification. Aim of this study was to evaluate Q-LAMP assay for detection of BKV in whole blood and urine specimens in comparison to Real-time assays.

#### METHODS

We tested 100 specimens (51 whole bloods and 49 urines) from immunocompromised patients previously tested in RT-PCR. For RT, viral DNA was extracted from 200µl of samples and was amplified with specific primers and probe for T antigen. For Q-LAMP assay, DNA was extracted from 250µl of urine and 200µl of whole blood mixed with Internal control (IC) and (for blood specimens) proteinase K.

#### RESULTS

BKV was detected by Real-time PCR in 16 samples of whole blood and in 31 samples of urine. The other specimens resulted negative for BKV. Q-LAMP BKV detected BKV DNA in 15 and 30 samples of blood and urine respectively. In 4 samples of blood and 2 samples of urine the test was considered invalid for the lack of amplification of IC. All the other samples resulted negative for BKV. For 4 samples (2 bloods and 2 urines) RT-PCR was negative and Q-LAMP . On the other hand 1 blood sample was positive in RT-PCR and negative in Q-LAMP. In all positive samples viral load detected by Q-LAMP was lower than in RT-PCR; except for one blood sample in which was higher.

#### CONCLUSIONS

These data show a good performance of Q-LAMP in detection of BKV even if it seems to underestimate the viral load. However, extraction kit has to be improved in order to reduce invalid results of amplification.

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# **EVALUATION OF FOUR COMMERCIAL EXTRACTION-QUANTIFICATION SYSTEMS TO MONITOR CMV VIRAL LOAD IN WHOLE BLOOD**

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## **BACKGROUND-AIM**

Measurement of cytomegalovirus (CMV) DNA viral load in the transplant patients has become a standard practice for monitoring the response to antiviral therapy. New platforms (Biomerieux and Siemens) have been recently commercialized to quantified CMV and must be compared to the previous assays.

## **METHODS**

Three extraction and real-time PCR amplification systems: Abbott m2000SP/RT (ABB), Biomerieux Emag/Estream (BIO), Siemens Versant kPCR (SIE) have been compared with our routine system Qiagen Qiasymphony RQO (QIA) for their capacity to quantify CMV DNA in whole blood samples. The 4 systems were tested on 10 negative samples, 10 replicates of an Internal Quality Control (IOC) for Intra-assay reproducibilities and 3 dilutions in triplicate of the World Health Organization (WHO) international standard. We also tested 57 samples from 25 patients allowing to obtained follows-up for 5 patients.

## **RESULTS**

None of the negative samples were amplified with the 4 platforms. The standard deviation for the IOC in Log UI/ml was QIA= 0.04, ABB=0.07, BIO=0.11, SIE=0.06 respectively. The mean of the 9 values of the WHO standard was (reference value = 4.7 Log UI/ml): QIA=4.84, ABB=4.61, BIO=4.33, SIE=4.79. One patient (10 samples) presented a major underquantification by QIA of 1.71 Log versus SIE, of 0.97 Log versus BIO and of 1.15 Log versus ABB.

On the 47 remaining samples with viral loads between 0 and 5.27 Log UI/ml with QIA (2 undetectable, 4 detected and 41 quantified), the qualitative analyze showed : Undetectable : ABB=4, BIO=6, SIE= 15, Detected : ABB=0, BIO=5, SIE=5. Three samples with an extraction problem or inhibited were not able to be quantified with BIO and SIE.

Between QIA and ABB, the difference of mean (DM) was -0.1 on Bland-Altman representation and the correlation coefficient was  $r=0.9219$ . Between QIA and BIO DM=-0.21 and  $r=0.8835$  and between QIA and SIE DM=-0.04 and  $r=0.7049$ .

## **CONCLUSIONS**

The intra-run reproducibility was very good for the 4 systems and the results obtained with the WHO standard were similar, except with BIO which was slightly lower. The results obtained on clinical samples with low viral loads were contrasted with very good results for QIA and ABB and a lack of sensitivity for SIE. One patient was also underquantified by QIA probably due to a divergent strain.

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# **EVALUATION OF FOUR COMMERCIAL EXTRACTION-QUANTIFICATION SYSTEMS TO MONITOR EBV VIRAL LOAD IN WHOLE BLOOD**

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## **BACKGROUND-AIM**

The Epstein Barr virus (EBV) DNA quantification in whole blood is an increasingly important marker which is used to predict risk of posttransplant lymphoproliferative disorders. New platforms (Biomerieux and Siemens) have been recently commercialized to quantified EBV and must be compared to the previous assays.

## **METHODS**

Three extraction and real-time PCR amplification systems: Abbott m2000SP/RT (ABB), Biomerieux Emag/Estream (BIO), Siemens Versant kPCR (SIE) have been compared with our routine system Qiagen Qiasymphony RQO (QIA) for their capacity to quantify EBV DNA in whole blood samples. The 4 systems were tested on 10 replicates of an Internal Quality Control (IOC) for the Intra-assay reproducibilities and on 3 dilutions in triplicate of the World Health Organization (WHO) international standard. We also tested 45 samples from 27 patients allowing to obtained follows-up for 3 patients.

## **RESULTS**

The standard deviation for the IOC in Log UI/ml was QIA=0.15, ABB=0.15, BIO=0.27, SIE=0.18 respectively. The mean of the 9 values of the WHO standard was (attended value = 4.7 Log UI/ml): QIA=4.70, ABB=4.61, BIO=4.62, SIE=4.57.

On the 45 samples with viral loads between 1.3 and 7.27 Log UI/ml with QIA (2 detected, 43 quantified), the qualitative analyze showed: Undetectable or detected: ABB=6 and BIO=4 (VL QIA 1.3 to 2.88 Log) and SIE=13 (VL QIA 1.3 to 3.97 Log). One sample inhibited was not able to be quantified with BIO. Four samples were detected or quantified only by QIA (VL between 1.3 and 2.88 Log).

Between QIA and ABB, the difference of mean (DM) was 0.16 on Bland-Altman representation and the Spearman's coefficient was  $r=0.900$ . Between QIA and BIO DM=-0.31 and  $r=0.878$  and between QIA and SIE DM=0.49 and  $r=0.649$ .

## **CONCLUSIONS**

The results obtained with the WHO EBV standard were similar between the 4 systems. On clinical samples with low viral loads the results were more contrasted with very good results for QIA, ABB and BIO. The four samples positive with QIA only could not be retested to exclude false positives results. The concordance between QIA and SIE is moderated; this may be explained by the fact that SIE is not yet CE-mark on whole blood.

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# **RAPID EMERGENCE OF DRUG-RESISTANT SYMPTOMATIC CONGENITAL CMV INFECTION: IMPACT OF DIFFERENT CANONICAL CMV UL97 DRUG RESISTANCE MUTATIONS AND PERSISTING LOW VIRAL LOAD**

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## **BACKGROUND-AIM**

About 10% of congenitally CMV (cCMV) infected infants have symptomatic CMV disease at birth, and about 35% show sensorineural hearing loss. A recent RCT-study documented that valganciclovir (VGCV) treatment of symptomatic cCMV infections for 6 months improved hearing and developmental outcome at 24 months modestly (Kimberlin et al., 2015). Only four case reports on the emergence of CMV drug resistance in cCMV are available.

## **METHODS**

A mature infant with cCMV was diagnosed on his first day of life due to blueberry muffin signs, severe hepatosplenomegaly, anemia and thrombopenia, chorioretinitis-scars bilaterally without impact on vision. Cerebral ultrasound showed typical periventricular calcifications. The child was immediately treated with iv ganciclovir. Due to impaired renal function with GFR around 25ml/kg/min dose adjustments were necessary, drug trough levels were too high several times. CMV UL97 genotyping from plasma, urine and throat swabs was initiated in week 7 p.p.

## **RESULTS**

A mature infant with severe cCMV is presented. After two weeks GCV iv, treatment with valganciclovir p.o followed. Based on low persistent CMV DNA levels, a longitudinally UL97 drug resistance monitoring from blood and urine was performed. 7 weeks after initiation of antiviral therapy, the UL97 mutation C603W was detected from blood. Interestingly, a switch to UL97 M460V and M460I mutations was observed in blood and urine two weeks later. In throat swabs, all three canonical UL97 mutations appeared and antiviral therapy was interrupted. During the antiviral therapy, levels of VGCV were always in the correct range. His further clinical course resulted in the need for bilateral cochlear implants due to deafness; he needed several transfusions due to profound anemia, substitution of fat-soluble vitamins and UDC due to hepatic involvement, and he has significant delay of motor functions so far at 6 months of age.

## **CONCLUSIONS**

Meanwhile the infant is deaf in both ears. This case demonstrates the failure of VGCV therapy despite correct levels of VGCV and persisting low level VL. Like in two other case reports (Campanini et al., 2012 and Choi et al., 2013) different canonical UL97 mutations were present simultaneously.

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# **INVESTIGATION OF ROUTINE DIAGNOSTIC HIV TESTING AS A TOOL TO DISTINGUISH RECENT FROM LONG-TERM INFECTION**

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## **BACKGROUND-AIM**

Accurate measurement of HIV incidence is required to identify populations at increased risk of HIV acquisition, to monitor the AIDS epidemic and to evaluate interventions for HIV prevention. Recently a routine diagnostic test was reported as a promising approach for staging HIV infections. The aim of this study was to compare two diagnostic platforms with our currently used Sedia HIV Limiting Antigen Avidity assay (LAG) for recency determination.

## **METHODS**

Newly confirmed HIV infections in 2016 were extracted from the National Virus Reference Laboratory (NVRL) HIV Database. Recent HIV infections were identified using the following criteria: Evidence of an HIV negative test in the previous 12 months, detection of p24 antigen on first diagnosis and application of the HIV INNO-LIA confirmatory assay banding pattern. All samples were tested using the LAG assay. The results of the LAG assay were compared using Pearson correlation and logistic regression analyses with two diagnostic platforms which use 4th generation assays; Abbott ARCHITECT and bioMerieux VIDAS®.

## **RESULTS**

Of the 455 new diagnoses, 110 were LAG recent. The cohort included 351 males and the age range was 16-72 years. HIV viral loads were significantly higher in males ( $p=0.011$ ). There was a strong and significant correlation between the ARCHITECT S/CO values and the LAG results,  $r=0.717$ ,  $p<0.001$ . Statistical analysis showed that the proportion of cases classified as LAG recent ( $ODn<1.5$ ) with ARCHITECT S/CO  $<50$  was 100% and S/CO  $<200$  was 91%. No samples with S/CO  $>400$  tested recent by LAG ( $ODn<1.5$ ). Using LAG as the 'gold standard,' our findings revealed that an Architect S/CO  $<400$  had a sensitivity and specificity of 90.32% and 89.83% respectively. There was a moderate and significant correlation between the VIDAS S/CO values and the LAG results,  $r=0.512$ ,  $p<0.001$ .

## **CONCLUSIONS**

In Ireland, the NVRL is the only laboratory which offers HIV avidity testing. The findings in this study show that a routine diagnostic platform, Abbott ARCHITECT, can provide accurate HIV staging in a subpopulation of patients with low or high S/CO values. The need for additional HIV avidity testing could therefore be decreased resulting in significantly reduced costs.

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# MOLECULAR TESTING FOR RELIABLE DIAGNOSIS AND INFECTION CONTROL OF RESPIRATORY VIRUS INFECTIONS: A STUDY COMPARING THE PERFORMANCE OF MANUAL AND FULLY AUTOMATED TESTING

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## BACKGROUND-AIM

Upper respiratory tract infections are highly prevalent in the average population during the winter season and most frequently caused by respiratory viruses, which can also cause severe lower respiratory tract infections in immunosuppressed patients. Rapid diagnosis is required as a basis for measures to control nosocomial infections of immunosuppressed patients. Therefore, Influenza and RSV monitoring of asymptomatic patients (for admission and on high risk wards) was implemented for the 2016/2017 season in our medical center, which cares for a large group of immunosuppressed transplant recipients. Moreover, panel virus diagnostics (Influenza, RSV, hMPV, Parainfluenza-, Rhino- and Adenovirus) triggered by symptoms was performed.

Diagnostic specimens and monitoring specimens were included in a study which compared the performance of the Fusion Panther respiratory panel real time PCRs with our routine diagnostic real time PCRs (in house assays for Adenovirus and Influenzavirus; Biomerieux R-Gene assays for hMPV, Parainfluenzavirus and Rhino/Enterovirus).

## METHODS

1675 specimens were included, both originating from the upper and the lower respiratory tract. More than 1400 of these specimens were tested for Influenza and RSV, about 50% originating from monitoring of asymptomatic patients. More than 500 specimens were tested for Adenovirus, Parainfluenzavirus, hMPV and Rhinovirus with about half of these originating from the lower respiratory tract.

## RESULTS

Results of both methods were >98% concordant for upper and lower respiratory tract specimens. Almost all discordantly tested specimens had high Ct values, thus only being faintly positive in one method. Ct values of both methods correlated very well (Spearman  $r > 0.9$ ). By monitoring of asymptomatic patients, detection of respiratory infections was feasible in the incubation period. Isolation of affected patients helped to prevent nosocomial outbreaks. Hands on time and turn around time (about 3.5h) were far shorter with the Fusion Panther system which is a fully automated and provides random-access.

## CONCLUSIONS

In conclusion, rapid diagnosis of respiratory virus infections is facilitated by the Fusion Panther system which provides a similar diagnostic performance compared to established real time PCRs.

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# IN VITRO AND IN VIVO INHIBITORY EFFECT OF CRUDE ETHYL ALCOHOL EXTRACT OF PEGANUM HARMALA L. SEED ON INFLUENZA VIRUS

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## BACKGROUND-AIM

Background and aim: Infections with influenza A virus is still a major health burden and the options for the control and treatment of the disease are limited. Therefore, this research was aimed to evaluate the antiviral effect of Peganum harmala L. (PHS) seed extract against influenza A virus both in vitro and in vivo.

## METHODS

Method: in vitro potential anti-influenza mouse-adapted Influenza virus (A/Puerto Rico/8/34, H1N1; PR8) activity of the extract was assessed using Madin-Darby canine kidney (MDCK) cells. For in vivo evaluation, four groups of female BALB/c mice were infected with the virus strain. Subsequently, infected mice in group 1 received no treatment, group 2 received the extract, group 3 received Oseltamivir phosphate (OP), and group 4 (without virus inoculation) received PHS extract orally. The virus titer in lungs of mice was determined using real-time qRT-PCR on day 3 post infection. The values between different groups were considered statistically significant when  $p < 0.05$ .

## RESULTS

Results: Based on our results, the CC50 and the IC50 value of PHS extract were  $124.6 \pm 5.1 \mu\text{g/ml}$  and  $16.35 \pm 3.7 \mu\text{g/ml}$ , respectively, with SI value of 7.62. Also, we showed that the extract significantly decreased the virus titer ( $p < 0.05$ ) and decreased the PCR products of influenza A virus M gene compared with those of the control ( $p < 0.05$ ). Our in vivo results showed that body weight loss was inhibited in the two groups of both PHS and OP infected mice compared with that of the virus control ( $p < 0.05$ ). Also orally administration of PHS and OP in the two groups of mice increased their survival rate compared with that of the control (virus) group ( $p < 0.05$ ). The PHS extract significantly reduced the viral load in the lungs of infected mice compared with that of the control (virus) group ( $p < 0.05$ ).

## CONCLUSIONS

Conclusion: Based on our findings, Peganum harmala seed extract inhibits influenza A virus replication both in vitro and in vivo. Therefore, it should be more characterized to be used as new herbal anti-influenza A virus agent.

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### ANTIVIRAL EFFECT OF SOLIDAGO VIRGAUREA AQUEOUS EXTRACT ON HERPES SIMPLEX VIRUS TYPE 1

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#### BACKGROUND-AIM

Herpes simplex virus type 1 (HSV-1), a worldwide prevalent pathogen, is the main cause of orofacial herpes, corneal blindness and several disorders of the peripheral and central nervous system. Acyclovir and other related drugs are used for treatment of HSV infections but the emergence of resistant viruses is a problem that urges for new solutions, especially in immunocompromised individuals, such as HIV infected patients and recipients of solid organ or bone marrow transplants [1]. Due to their low side effects and costs of production, many antiherpetic assays have focused on medicinal plants.

#### METHODS

We evaluated the anti-herpetic effect of an aqueous extract (AE) from stems/leaves of *Solidago virgaurea* L. (Asteraceae) that has been previously characterized by HPLC-DAD and LC-MS analyses, revealing the presence of flavonoids and caffeic acid derivatives [2]. All the assays were made in Vero E6 cultures infected with HSV-1 and treated with non-cytotoxic concentrations of the AE. The virucidal effect was assessed by comparison of the titers of virus particles incubated in the presence/absence of AE. The effect on HSV-1 replication cycle was evaluated by treatment of cells during virus titration and by treatment of infected cells throughout viral production in a single replication cycle. In both cases, infected non-treated cells and infected cells treated with acyclovir were used as negative and positive controls, respectively.

#### RESULTS

A reduction of more than 90 % in the titer of HSV-1 suspensions produced in treated versus non-treated infected cells is observable.

#### CONCLUSIONS

As previously reported for HSV-2 [3], these results also suggest that *S. virgaurea* AE have inhibitory activity on the HSV-1 replication cycle, hence showing once more the potential use of this extract in the treatment of infections caused by Herpes simplex viruses.

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### EVALUATION, INCLUDING THE APPLICABILITY OF INTERNAL CONTROLS, IS NEEDED PRIOR TO APPLICATION OF ROUTINE HIGH RISK-HUMAN PAPILLOMAVIRUS TESTS ON SELF-COLLECTED SAMPLES

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#### BACKGROUND-AIM

Self-collection for high risk human papillomavirus (hr-HPV) is a trending topic in primary cervical cancer screening. However, technical formalities like validation of a specific sample type, are often disregarded as a source of variability in (study) results. This study evaluates applicability of a certain self-sampling device on our routine molecular procedures for hr-HPV detection.

#### METHODS

In a primary health care facility in Kinshasa, Congo, 187 self-collected samples (Evalyn Brush) were gathered and sent to Ghent University Hospital (UZ Gent) and Algemeen Medisch Labo (AML) where routine tests for hr-HPV were applied (Abbott RealTime High Risk HPV and qPCR (E6/E7) respectively). Sample type effect is evaluated by comparing the internal control (IC) in self-collected samples and in clinician-taken samples randomly selected from UZ Gent archive.

#### RESULTS

In UZ Gent an error was found in 9.1% (17/187) due to lack of IC signal. The hr-HPV prevalence in the remaining 170 samples was 18,8% (32/170). HPV16 infection was seen in 5/32 (15,7%). In two cases a co-infection between HPV18 and another hr-HPV type was seen (6,3%) and the remaining positive samples 25/32 (78,1%) had an infection with one or more other hr-HPV types included in the assay. Comparing IC results between the self-collected and clinician-collected group, a significant difference ( $p < 0,001$ ) was found, with higher IC signals (or lower CT values) in the clinician-collected group. In AML an error was encountered in 17.6% (33/187) samples, including 16/17 of the UZ Gent. The remaining UZ Gent IC- error gave a negative result in AML. In the remaining 154 samples a concordance of 90% was seen between both laboratories with a 77% negativity rate.

#### CONCLUSIONS

In our study, a high IC-error rate was encountered ranging from 9.1% to 17.6% on self-collected specimens. The possible explanation for this finding can be a) not adjusted cut-off's for self-samples or b) pre-analytical effect related to inadequate sampling strategy. Evaluation of test used, including the applicability of IC, for self-collected specimens have to be performed prior to its actual implementation in the laboratory.

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**COMPARISON OF THE APTIMA HIV-1, HCV AND HBV QUANT DX ASSAYS WITH THE HIV, HCV AND HBV ABBOTT REAL TIME ASSAYS**S. May<sup>1</sup>, E. Adamska<sup>1</sup>, J. Tang<sup>1</sup><sup>1</sup>University Hospital Leicester**BACKGROUND-AIM**

The Aptima HIV-1 Quant Dx, HCV Quant Dx, and HBV Quant assays utilise the transcription-mediated amplification (TMA) method for the detection and quantification of HIV-1 RNA, HCV RNA and HBV DNA. The aim of this study was to assess the performance of the Aptima TMA assays and compare these to the real time Abbott HIV-1, HCV and HBV assays.

**METHODS**

For each of the assays correlation between quantitative results of Aptima and Abbott assays, as well as accuracy and reproducibility of the Aptima assays were assessed. Testing was also performed on the 3rd WHO HIV-1 international standard, the 2nd WHO international reference panel for HIV-1, the 5th WHO international standard for HCV NAT testing, the NIBSC 4th HCV RNA panel, and the 3rd WHO HBV international standard. Patient samples known to be positive for HIV, HCV or HBV were selected. Samples with a range of subtypes/genotypes and viral loads were tested.

**RESULTS**

Comparisons of each of the pairs of assays found that they were highly correlated (HIV:  $R^2 = 0.915$ , HCV:  $R^2 = 0.97$  and HBV:  $R^2 = 0.94$ ). Lin's concordance coefficients were 0.93, 0.81 and 0.95 for HIV, HCV and HBV respectively. Mean differences of measurement according to the Bland-Altman method were 0.196 copies/ml, 0.011 IU/ml and -0.317 IU/ml for HIV, HCV and HBV respectively. The HIV Quant dx assay detected the WHO international standard from 4.27 down to 1.26 log<sub>10</sub> copies/ml with good linearity ( $R^2 = 0.96$ ) and also detected all subtypes from the 2nd WHO international reference panel. The HCV Quant dx assay detected the 5th WHO international standard from 4 down to 1 log<sub>10</sub> IU/ml with good linearity ( $R^2 = 0.95$ ) as well as all genotypes in the NIBSC 4th HCV RNA panel. The HBV Quant assay detected the 3rd WHO HBV international standard from 4.93 log<sub>10</sub> IU/ml to 0.90 log<sub>10</sub> IU/ml with good linearity ( $R^2 = 0.99$ ).

**CONCLUSIONS**

The Aptima HIV-1, HCV Quant Dx and HBV Quant assays demonstrated comparable performance to the Abbott assays for the detection and quantification of HIV-1, HCV and HBV viruses.

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**ANALYTICAL PERFORMANCE OF THE HOLOGIC APTIMA HBV QUANT ASSAY AND THE ROCHE COBAS AMPLIPREP/COBAS TAQMAN HBV TEST V2.0 FOR THE QUANTIFICATION OF HBV DNA IN PLASMA SAMPLES**K. Schønning<sup>2</sup>, K. Johansen<sup>1</sup>, L.G. Nielsen<sup>1</sup>, H. Westh<sup>2</sup><sup>1</sup>Department of Clinical Microbiology, Hvidovre Hospital, Hvidovre, Denmark.<sup>2</sup>Department of Clinical Microbiology, Hvidovre Hospital, Hvidovre, Denmark; Institute for Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.**BACKGROUND-AIM**

Quantification of HBV-DNA in serum or plasma is used as decision support for initiating, monitoring and stopping virologically failing antiviral treatment. Analytical performance of different tests may therefore impact clinical treatment decisions. In the present study we compared the analytical performance of the Aptima HBV Quant Assay (Aptima) and the COBAS Ampliprep/COBAS TaqMan HBV Test v2.0 (CAPCTM) for the quantification of HBV DNA in plasma samples.

**METHODS**

The two tests were evaluated on 129 prospective plasma samples and on 63 archived plasma samples, of which 46 had been genotyped. Furthermore, linearity and limit of detection were assessed using dilution series of three clinical samples (Genotype B, C, and D).

**RESULTS**

Of the 192 clinical samples, 105 quantified with a result > Log 2 IU/mL in both tests. Results obtained on prospective and archived samples were compared using Deming regression analysis. As the regression lines of the two data sets did not differ significantly, the two data set were consolidated. The consolidated Deming analysis showed the two tests correlated excellently (slope of the regression line 1.00, 95%CI: 0.966-1.041;  $r = 0.982$ ). Mean bias in quantification between the two tests (Aptima – CAPCTM) was -0.15 Log IU/mL (SD: 0.31), and only minor differences were observed for samples with a defined genotype (genotype B (N=7; average difference: -0.13 IU/mL), C (N=7; average difference: -0.31 IU/mL), and D (N=22; average difference: -0.22 IU/mL). Four samples quantified more than two standard deviations higher in Aptima than in CAPCTM. Both tests were sensitive and precise with %CV less than 2% for HBV DNA concentrations >3 Log IU/mL.

**CONCLUSIONS**

The Aptima assay and the CAPCTM test are highly correlated. Both tests are sensitive and precise. Linearity and inclusivity of the Aptima test is excellent and the test is useful to monitor antiviral treatment in HBV infected individuals.

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### DETECTION AND CHARACTERIZATION OF HUMAN DNA VIRUSES IN FEMORAL BONE

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#### BACKGROUND-AIM

Several human DNA viruses are known to persist in soft tissues long after primary infection. Yet, little is known about viral persistence in bone. It is here however, where the viral DNA is most likely to be preserved across time. Thus, bone may withhold an invaluable window to the past, providing evidence on viral origin, evolution and adaptation.

We have detected and characterized DNA of human parvovirus B19 in 7-decade-old human skeletal remains. In the present study, we aim to characterize the full set of human DNA viruses that persist in femoral bone to determine the basis for work on ancient human remains.

#### METHODS

To assess the prevalence of human DNA viruses in bone of the present-day population, samples from cortical femoral bone (outer and inner surface samples) and of bone marrow were collected from 30 recently deceased individuals.

The DNA was extracted using two methods of known efficiency for DNA analysis from bone (Dabney et al 2013 PNAS and Toppinen et al 2015 Sci Rep). We here validated these methods for their suitability for viral DNA.

The bone samples were screened for persisting viral DNA by quantitative PCRs not only for comparison between extraction methods but also for reference means of superior sensitivity to other approaches such as next generation sequencing (NGS). For correlation of the viral loads to the number of cells, all samples were assessed for single copy human RNase P gene. Moreover, the viral populations will be investigated using targeted enrichment with virus-derived biotinylated RNA baits. After hybridization, the viral DNA fragments present in the samples are captured with streptavidin-coated magnetic beads, amplified and sequenced by NGS.

#### RESULTS

Preliminary results show that indeed viral DNA can be detected in human femoral bone, including for example sequences belonging to the families Parvoviridae, Anelloviridae and Herpesviridae. The validation of the extraction methods of viral DNA from bone is ongoing.

#### CONCLUSIONS

We here demonstrate that viral nucleic acids can be detected in and genetically characterized from human bone. Such material as well as older skeletal specimens are thus suitable for investigations of epidemiologies and evolutionary histories of a range of DNA viruses.

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### INVESTIGATION OF ATYPICAL SEROLOGICAL PROFILES FOR EPSTEIN BARR VIRUS

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#### BACKGROUND-AIM

Commercial immunoassays that detect IgG and IgM against Epstein-Barr virus (EBV) viral capsid antigens (VCA), and IgG toward EBV nuclear antigen (EBNA) are routinely used in combination to categorize EBV infection status. However, this strategy does not always allow to confirm/exclude recent/past EBV infection. Our aim was to perform complementary investigations when these atypical serological profiles were observed.

#### METHODS

In our laboratory, using IgG/IgM EBV LIAISON XL® DiaSorin assay, we observe atypical EBV serological profiles in 18.3% cases. Complementary investigations performed on 595 samples included: CMV IgG/IgM/IgG avidity (LIAISON XL®, DiaSorin), Immunoblot (Ib) EBV IgG/IgM (Mikrogen®), EBV PCR (QIASymphony® SP QIAGEN, Rotor-Gene Q). EBV PCR was performed in order to confirm/exclude an active infection. Ib and CMV serology were performed in order to evaluate specificity/sensitivity of the IgG/IgM EBV LIAISON XL® DiaSorin assay.

#### RESULTS

Results for the different atypical EBV serological profiles were:

- Positive IgG anti-EBNA alone (2.6% N=84) : Ib IgG positive (pos) in 66% cases (past infection), PCR/Ib negative (neg) 23% (no immunity), PCR pos 11% (reactivation) ;
- IgM/IgG anti-VCA and IgG anti-EBNA positive (2.3% N=76) : PCR neg in 51% cases (nonspecific stimulation polyclonale of the immune system), PCR pos 33% (reactivation), CMV primary infection 16% (cross reaction) ;
- Positive IgM anti-VCA alone (0.8% N=25) : Ib IgM/PCR neg in 52% cases (nonspecific IgM), PCR pos 42 % (EBV primary infection), CMV acute infection 4% (cross reaction) ;
- Positive IgG anti-EBNA and positive IgM anti-VCA (0.5% N=16) : Ib IgG-IgM/PCR neg in 47% cases (nonspecific reactivity), Ib IgM/PCR neg and Ib IgG pos 33% (past infection), Ib IgM/PCR pos and Ib IgG neg 20% (EBV acute infection);
- Positive IgG anti-VCA alone (12.1% N=394): Ib IgG/PCR neg in 5% cases (nonspecific reactivity), Ib IgG/PCR pos 20% (active infection), Ib IgG pos and PCR neg 75% (past infection).

#### CONCLUSIONS

Atypical EBV serological profiles may be due to several clinical situations, and complementary investigations seem important in order to contribute rapidly to the diagnostic, and avoid additional sample collection which is usually not easier to interpret.

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### THE USE OF DRIED BLOOD SPOTS (DBS) TO DETERMINE HEPATITIS C VIREMIA AND GENOTYPE IN A HIGH-RISK POPULATION

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#### BACKGROUND-AIM

Tackling chronic, active hepatitis C virus (HCV) infection has been identified as a strategic priority for the WHO, with a global target of elimination of HCV by 2030. Increased awareness and screening, along with the upscale of highly effective treatment with direct acting antivirals, are all important components of this elimination strategy. Identification of infectious hepatitis C in at risk, yet hard-to-reach populations is currently advocated to facilitate enhanced surveillance and prevent onward transmission. Screening with dried blood spots (DBS), using finger-prick blood, combines ease of testing in a community setting and therefore is an ideal tool for identifying individuals who may refuse venepuncture. Expansion of the test repertoire of this screening tool to include detection of HCV RNA and genotype, from the initial sample, would be an additional advantage as all the relevant results of those with active HCV would be known at the time of referral.

#### METHODS

As part of the National Opt-Out BBV testing Programme for Prisons in England, screening with DBS was piloted in 3 prisons nationwide. Samples were eluted for routine HIV, Hepatitis B and HCV antibody testing on an automated platform. In addition, qualitative HCV RNA detection, as well as genotyping, was determined using Abbott RealTime HCV and HCV Genotype II assays from the same DBS sample.

#### RESULTS

From November 2015 to end of February 2017, 1904 inmates were screened for blood-borne viruses, with 8.6% found to be HCV antibody positive, 1.2% positive for Hepatitis B surface antigen and 0.2% positive for HIV antibody. Interestingly, among the HCV positive inmates, 76.7% were found to be viremic, with genotype 1a predominant. These results are broadly reflective of the prevalence, determined using venepuncture sampling, in the prison population of the UK.

#### CONCLUSIONS

DBS is a suitable sample for determining active HCV and genotype and is therefore an ideal candidate screening tool in hard to reach populations.

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### SPECIFIC BIOMARKERS FOR RABIES ANTEMORTEM DIAGNOSIS: RECENT ADVANCES

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#### BACKGROUND-AIM

Our target of our communication is to prove the potential of the identification of specific rabies biomarkers in the revolution of rabies diagnostic and therapy in future.

#### METHODS

Samples: human brain tissues from confirmed cases of encephalitic and paralytic rabies cases, vaccinated and uninfected normal brain tissue

Analysis method: iTRAQ-based quantitative proteomic using high resolution mass spectrometry to identify candidate biomarkers in rabies

Validation method:

-immunohistochemistry. And dot blot assays

-ELISA,MRM,Mass Spectrometry

#### RESULTS

402 proteins associated with rabies

39 proteins as differentially regulated in encephalitic rabies, 46 proteins in vaccinated rabies, 39 proteins in paralytic rabies (KPNA4) overexpressed only in paralytic rabies, (CAMK2A) which was overexpressed only in paralytic rabies,(GLUL) which was overexpressed in paralytic as well as encephalitic rabies

#### CONCLUSIONS

confirmation of specific rabies biomarkers allows Rapid diagnosis of rabies which is vital for initiating prompt and appropriate infection control and public health measures. Early diagnosis can obviate the need for unnecessary treatment and medical tests and also help in prognostication, institution of barrier nursing, timely administration of pre or postexposure prophylactic vaccination to family members of the patient and the treating medical and nursing staff, and case closure and grief counseling. Laboratory tests negative for rabies can indicate the presence of another infectious agent or a noninfectious etiology, and assist in appropriate medical management. Laboratory diagnosis of rabies can also help specific characterization of the causative agent and suggest the potential source of infection

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# IN VITRO ANTI-ADENOVIRAL ACTIVITY OF CRUDE ETHANOL EXTRACT AND FOUR CORRESPOND FRACTIONS OF POMEGRANATE (PUNICA GRANATUM L.) PEEL EXTRACT

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## BACKGROUND-AIM

Background-aim: Adenovirus causes a number of diseases in human, and to date, no specific antiviral therapy is approved against this virus. Thus, searching for effective anti adenovirus agents seems to be required. Many studies have shown that components derived from medicinal plants have antiviral activity. Therefore, this study was aimed to evaluate in vitro anti adenovirus activity of crude ethanol extract and four correspond fractions of pomegranate peel.

## METHODS

Methods: In this experimental research, crude ethanol extract of Pomegranate (*Punica granatum* L.) peel was prepared and subjected to fractionation with different polarity. Anti-adenovirus activity of the extract and fractions was evaluated on HEp2 cell line using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The 50% inhibitory concentration (IC50) and 50% cytotoxicity concentration (CC50) of the extract/fractions were determined using regression analysis and from which selective index (SI), the CC50/IC50 ratio was calculated. Its inhibitory effect on adsorption and/or post-adsorption stages of the virus replication cycle was evaluated.

## RESULTS

Results: Based on our results, there was significant relationship between the concentration of Pomegranate extract and the correspond fractions and both cytotoxicity and cytopathic effect (CPE) inhibition ( $P < 0.05$ ). The crude extract and n-Butanol fraction had the highest inhibitory effect against adenovirus with IC50 value of 5.57 (CI95%:4.9-6.2), and 2.16 (CI95%: 1.7-2.6) and selectivity indices of 37.9 and 113.3, respectively. The crude extract and n-Butanol fraction inhibited the virus replication at post-adsorption stage ( $p < 0.01$ ).

## CONCLUSIONS

Conclusion: Pomegranate peel extract and particularly the correspond n-Butanol fraction with high inhibitory effect against adenovirus replication in vitro might be considered as herbal anti-adenovirus agents. Also, this extract with high phytoconstituents could be a promising source of medicinally important natural compound

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# COMPARISON OF THE BD PROBETEC HERPES SIMPLEX VIRUSES (HSV 1 AND 2) QX AMPLIFIED DNA ASSAY AND THE APTIMA HSV 1 AND 2 ASSAY ON VTM ANOGENITAL SWABS

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## BACKGROUND-AIM

The HSV-1 and 2 are enveloped, double-stranded DNA viruses belong to the Herpesviridae. People are usually infected as young children and remain infected for life. The viruses persist in infected hosts as mostly asymptomatic latent infections, with occasional symptomatic reactivation (e.g. cold sores and genital ulcers). Historically, herpesvirus infections were diagnosed using viral isolation then type-specific immunofluorescent antigen detection, but now nucleic acid detection methods are the norm.

## METHODS

This study compared the BD Probetec Herpes Simplex Viruses (HSV-1 and 2) Qx Amplified DNA (strand displacement) assay on the BD Viper, currently in-service, with the Aptima (transcription-mediated amplification of mRNA) HSV-1 and 2 assay on the Panther. A total of 255 genital swabs and 2 rectal swabs in virus transport medium were used in this evaluation. Each of the samples was tested on both the BD viper and Aptima assays, with discrepant samples additionally tested on an in-house PCR assay.

## RESULTS

Discrepant results were seen for 7.7% (n=20) of the samples tested on both assays, with the rest being concordant. The sensitivities of the Aptima and BD probetec assays were respectively: 91% and 94% for HSV-1; 85% and 98% for HSV-2. Similarly, the specificities of the Aptima and BD probetec assays were respectively: 100% and 98% for HSV-1; 100% and 99% for HSV-2. The intra- and inter-run reproducibility for the Aptima assay was 100% for both HSV-1 and HSV-2.

## CONCLUSIONS

The BD probetec assay was more sensitive for the detection of HSV-1 and HSV-2, whereas the Aptima HSV-1 and 2 assay was found to be more specific. Samples run on these assays are usually taken from patients attending sexual health clinics. These patients are generally treated empirically at their first visit, based on a clinical assessment alone. Laboratory-confirmed results for HSV-1 or 2 positive lesions are useful when considering the need for longer-term suppressive therapy. Thus, a patient tested with a less sensitive test (i.e. with more false negatives) may experience delays in commencing longer-term suppressive therapy. Whereas a patient tested with a less specific test (i.e. with more false positives) may experience unnecessary longer-term suppressive therapy. However, all patients are reassessed at each visit.

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**CMV DNA VIRAL LOAD IN WHOLE BLOOD WITH DXN VERIS**A. Nava<sup>1</sup>, S. Torri<sup>1</sup>, M. Oggioni<sup>1</sup>, V. Lepera<sup>1</sup>, D. Fanti<sup>1</sup><sup>1</sup>S.C. Analisi Chimico Cliniche e Microbiologia- ASST Grande Ospedale Metropolitano Niguarda-Milano**BACKGROUND-AIM**

Monitoring of CMV is fundamental for starting pre-emptive therapy in transplant patients, therefore a reduction in Turn around time (TAT) is desirable for a rapid treatment. CMV DNA viral load can be performed on both whole blood and plasma. The Beckman Coulter® DxN VERIS platform is a fully automated, moderate complexity, random-access, sample-to-answer system for the quantitative/qualitative analysis of molecular targets. DxN VERIS has the CE-IVD mark for plasma testing, therefore the aim of our study was the evaluation of its performances on whole blood.

**METHODS**

We analysed VERIS sensitivity, specificity, linearity, precision, patients monitoring and method comparison relative to Abbott M2000 Real-time assay.

**RESULTS**

The specificity was evaluated on 20 negative samples repeated for 3 days; it was 98,3%. Beckman Coulter claims a lower limit of detection (LOD) for plasma of 31.2 UI/ml; we spiked negative whole blood with WHO standard at a concentration of 100, 50, 25, 12 UI/ml. The probit analysis showed a LOD of 26.2 UI/ml. We tested 138 whole blood positive samples with DxN VERIS and Abbott M2000, 1 sample was detected under the limit of quantification (LLO) for both platforms, 27 were quantified by Abbott M2000 (LLO 31 UI/ml) and were detected with VERIS (LLO 120 UI/ml), and 110 samples were quantified by both systems, which were used for the methods comparison analysis. The Passing-Bablok fit showed a correlation of 0.666 between results; the Bland-Altman plot showed a broad dispersion of the data but only 5 samples have a difference in quantification higher than 1 Log. The average deviation was -0.15 logcp/ml for VERIS platform relative to M2000. We monitored 4 patients (4 time point each): VERIS results were comparable with the ones of M2000.

**CONCLUSIONS**

Our results suggest that DxN VERIS has a good sensitivity, specificity, linearity and precision for CMV DNA viral load testing on whole blood. Beckman Coulter CMV assay is comparable with Abbott M2000 CMV Real-time assay. VERIS can increase the productivity of molecular laboratories because it allows the continuous loading of single samples for different assays, eliminating batching and reducing TAT.

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**THE BASIS OF VIRAL SAFETY OF TRANSFUSION MEDICINE: SCREENING OF DONOR BLOOD AND METHODS OF VIRUS INACTIVATION**N. Shurko<sup>1</sup>, V. Novak<sup>1</sup>, T. Danysh<sup>1</sup><sup>1</sup>State Institution «Institute of Blood Pathology and Transfusion Medicine NAMS of Ukraine», Lviv**BACKGROUND-AIM**

The range of medicinal perpetrators are manufactured from pools of human blood. Modern medications should only be on high-purity, but also ensure high viral safety.

The selection of donors, sampling and screening of blood is the key step in the production process of plasma concentrate. That is why is so important to ensure proper compliance of this process. The next important step on the viral safety is of antiviral treatment methods.

Aim: to analyze the frequency dissemination of viral infection among donors Ukraine and investigate the use of ammonium thiocyanate in the manufacturing process of concentrate coagulation factor VIII.

**METHODS**

The paper describes the dissemination of transfusion infections among donors and research the possibility of using ammonium thiocyanate (NH<sub>4</sub>SCN) antiviral treatment of plasma. We use Immunate was initial raw material; methods - one-stage clotting method and ultrafiltration.

**RESULTS**

The modern viral safety of factor VIII concentrates dramatically improved the treatment and quality of life of hemophilia's patients.

The first stage of blood collection and screening were rejected certain group (calculated per 100,000 donations): 108.50±0.09 (0,11 %) (HIV); 947.8±1.0 (0,95 %) (HBB); 1028.7±1.0 (0,10 %) (HBC); 942.2±0.9 (0,94 %) (Syphilis). These samples were immediately withdrawn from plasma fractionation.

Then after follow certain manufacturing procedures pool of plasma is directed to the production of concentrate. The next stage of investigated was the possibility of using of NH<sub>4</sub>SCN in the technological scheme of production of concentrate factor VIII.

In the reaction mixture was added a solution of NH<sub>4</sub>SCN various concentrations. Removing was performed in stages by ultrafiltration. We shown that effect of NH<sub>4</sub>SCN the activity of factor VIII was the reverse and might used to antiviral treatment of factor coagulation VIII.

**CONCLUSIONS**

The implementation of viral inactivation techniques for the production of plasma-derived factor concentrates, as well as the adoption of new methods to screen viruses in blood donations, greatly improved the safety of plasma-derived products.

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**IMPROVED DETECTION OF HEPATITIS C VIRUS (HCV) RNA WITH THE NOVEL ARTUS® HCV QS-RGQ KIT V2**U. Siemetzki-Kapoor <sup>2</sup>, J. Gibson <sup>2</sup>, S. Ma <sup>2</sup>, D. Tomlinson <sup>2</sup>, I. Kavanagh <sup>2</sup>, G. Wall <sup>1</sup><sup>1</sup>QIAGEN GmbH<sup>2</sup>QIAGEN Ltd**BACKGROUND-AIM**

Since the advent of new Direct Acting Antiviral therapies for HCV, a cure is now possible for the vast majority of patients. Nucleic acid amplification tests (NAATs) are important tools in monitoring the response to antiviral therapy and are able to provide a surrogate marker of cure at the end of treatment. As a consequence, the high sensitivity of an HCV viral load assay becomes increasingly important, as referenced in clinical management recommendations such as those from EASL or AASLD.

The novel artus HCV QS-RGQ Kit v2 is a quantitative, real-time PCR-based assay currently under development for the detection and quantification of HCV RNA in EDTA plasma from individuals undergoing antiviral therapy in line with aforementioned recommendations. The assay runs on the automated QIAasymphony® SP/AS and Rotor-Gene® Q instruments. This study evaluated the performance characteristics during the development phase of the artus HCV QS-RGQ Kit v2.

**METHODS**

To assess the performance characteristics of the artus HCV QS-RGQ Kit v2, a series of studies were conducted. Limit of Detection (LoD) and Limit of Quantification (LoQ) studies were conducted using the HCV 5th WHO International Standard as well as clinical samples representing HCV genotypes 2–6.

**RESULTS**

The artus HCV QS-RGQ Kit v2 is designed to detect genotypes 1–6 and confirmed subtypes. Preliminary Limit of Detection studies demonstrated that all 6 HCV genotypes can be detected with a 95% hit rate at 15 IU/ml. Estimation of LoQ values based on Total Analytical Error (TAE) calculation suggest a Lower Limit of Quantification (LLoQ) equal to the estimated LoD (15 IU/ml).

**CONCLUSIONS**

The artus HCV QS-RGQ Kit v2 demonstrated high analytical performance and broad inclusivity of HCV strains and subtypes tested.

Keywords: HCV, artus, QIAasymphony

Disclaimer: The artus HCV QS-RGQ Kit v2 is currently under development and not commercially available.

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**PERFORMANCE OF THE ARTUS® CMV QS-RGQ MDX KIT**U. Siemetzki-Kapoor <sup>2</sup>, C. Kong <sup>2</sup>, G. Wall <sup>1</sup>, D. Tomlinson <sup>2</sup>, I. Kavanagh <sup>2</sup><sup>1</sup>QIAGEN GmbH<sup>2</sup>QIAGEN Ltd**BACKGROUND-AIM**

The artus CMV QS-RGQ MDx Kit is an in vitro nucleic acid amplification test for the quantitation of human cytomegalovirus (CMV) DNA in human EDTA plasma and is intended for use as an aid in the management of solid organ transplant patients undergoing anti-CMV therapy.

**METHODS**

To assess the analytical performance characteristics of the artus CMV QS-RGQ MDx Kit, a series of studies were conducted using either the 1st WHO International Standard for CMV or clinical samples representing CMV genotypes gB2, gB3 and gB4. Performance was assessed by testing paired CMV positive and CMV negative clinical samples with the artus CMV QS-RGQ MDx Kit and the manual artus CMV RGQ MDx Kit (EZ1/RGQ). Additional samples were prepared by spiking either a secondary standard or culture supernatant into EDTA plasma in order to obtain mid to high concentrations of the linear range and to cover all common CMV genotypes.

**RESULTS**

The artus CMV QS-RGQ MDx Kit demonstrated equal performance when compared to the artus CMV RGQ MDx Kit with an LOD of 67 IU/ml for the 1st WHO Std and 77 IU/ml for gB2, gB3 and gB4. The assay was shown to be linear from 119.1 to 7.94x 10<sup>7</sup> IU/ml. Several common pathogens and interfering substances (endogenous and exogenous) were tested and demonstrated no interference on the determination of the CMV load. At 3x LOD the repeatability and intermediate precision of the artus CMV QS-RGQ MDx Kit demonstrated SD values of 0.226 and 0.228 log<sub>10</sub> IU/ml, respectively. At the medical decision point (1000 IU/ml) the SD values were 0.077 and 0.096 log<sub>10</sub> IU/ml, respectively. In the study comparing the QS-RGQ MDx workflow with the manual EZ1/RGQ workflow, 97.59% of the clinical samples fell within the Allowable Total Difference (ATD) zone. Of the contrived samples spanning the mid to high end of the linear range, 100% fell within clinically relevant limits of ±0.5 log<sub>10</sub> IU/ml. Qualitatively, the Overall Percent Agreement between both the workflows was 94.31%.

**CONCLUSIONS**

The artus CMV QS-RGQ MDx Kit demonstrated comparable overall performance to the artus CMV RGQ MDx Kit, making this assay a suitable option for assessing the response to antiviral drug therapy.

Disclaimer: The artus CMV QS-RGQ MDx Kit and the artus CMV RGQ MDx Kit are FDA approved in the USA.

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### THE IMPACT OF ELITE INGENIUS™: A FULLY AUTOMATED SAMPLE-TO-RESULTS SOLUTION FOR CLINICAL LABORATORY ROUTINE

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#### BACKGROUND-AIM

In recent years virology laboratories undergo a constant increase in workload for molecular testing in terms of test numbers, assay types and specimens matrix, all combined with need of TAT (Turn Around Time) reduction. ELITE InGenius™ is a fully-automated, sample-to-result system of moderate complexity, for the quantitative/qualitative analysis of different molecular targets and matrices.

#### METHODS

The routine method consisted of automatic extraction by easyMAG, manual setup of amplification plate using ELITech reagents, amplification by 7500 Fast Dx Real Time PCR Instrument, raw data analysis and results interpretation, whereas InGenius combines all these steps. We evaluated reagents consumptions, working steps and TAT of different targets on both systems for 2 months.

#### RESULTS

We compared our routine method with ELITE InGenius™ system that shows low complexity of use: only 9 different consumables and 10 working steps were necessary to operate. In contrast 16 consumables and 19 working steps for routine manual runs. The results were available in 2h 50min (11.7% manual and 88.3% automation) with InGenius™ and in 3h 40min (36.4% manual) with the routine method. During one week we processed 12 cerebrospinal fluids (CSF) for quantification of different viral targets and 36 whole blood or plasma samples for EBV quantification. The reporting TAT for 10 CSFs was 12h and 2 within 24h with InGenius™. By the routine method 1 CSF only was reported within 12h; 7 CSFs in 24h and 4 CSFs in 48h-72h. Similarly, with InGenius™ the results of EBV testing were all available within 24h from arrival, whereas with the routine method only 15 samples were reported in 24h. The routine method required a longer TAT because of the sample batch testing (2 runs a week) needed to optimize costs. Moreover, in two months for 1200 requested analysis, the use of additional tests for calibration and controls was reduced with InGenius™ compared to the routine method (each run needed new calibration and controls): 120 versus 800.

#### CONCLUSIONS

ELITE InGenius™ is simple to use and requires limited hands-on-time. It's high flexibility allows to analyze single samples with different assays thus reducing TAT for urgent samples such as CSF and monitoring of transplanted patients.

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### HUMAN CYTOMEGALOVIRUS-SPECIFIC MEMORY T-CELL RESPONSE AND ITS CORRELATION WITH VIRUS TRANSMISSION TO THE FETUS IN PREGNANT WOMEN WITH PRIMARY INFECTION

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#### BACKGROUND-AIM

Primary Human cytomegalovirus (HCMV) infection during pregnancy is the major cause of congenital viral infection. The HCMV-specific T-cell response may have a role in the prevention of virus transmission to the fetus.

#### METHODS

HCMV-specific memory T-cells were investigated in 44 pregnant women (15 of whom transmitted the infection to the fetus) within 1-2 months after onset of primary infection. For comparison, 8 pregnant women with remote infection were analyzed. PBMC were stimulated for 12 days with overlapping 15-mer peptide pools of HCMV proteins immediate early (IE)-1, IE-2 and phosphoprotein (pp) 65, and subsequently re-stimulated with the corresponding peptide in a cultured-ELISPOT assay.

#### RESULTS

In remote infections no significant difference was observed among T-cell responses to pp65, IE-1 and IE-2. Instead, in primary infection, the pp65-specific T-cell response was significantly greater with respect to IE-1 and IE-2 ( $p < 0.05$ ). However, the response to all three proteins was significantly lower in primary infection with respect to remote infection ( $p < 0.05$  for pp65,  $p < 0.01$  for IE-1 and IE-2). In primary infection, expandable T-cells directed to pp65 and, when detectable, to IE-1 were predominantly CD4+. Strikingly, the response to pp65 was significantly lower ( $p < 0.01$ ) in women transmitting the infection to the fetus. A cultured ELISPOT response  $> 20$  was associated with an odds ratio of 7.14 (95% CI 1.65 to 30.89) for non-transmission of the virus to the fetus. To detect other factors potentially associated with non-transmission, different serological parameters were analyzed. Only IgG avidity index was higher in non-transmitting mothers, who showed also a lower DNAemia level. These two parameters remained associated with congenital infection in multivariate analysis.

#### CONCLUSIONS

Determination of HCMV-specific T-cells by cultured-ELISPOT, in pregnant women with primary HCMV infection in association with avidity index and DNAemia may help to assess the risk of HCMV fetal transmission.

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**A CONTINUOUS YEAR-ROUND INCIDENCE OF  
VARICELLA IN CZECH REPUBLIC**P. Bostik<sup>1</sup>, V. Bostikova<sup>1</sup><sup>1</sup>Faculty of Military Health Sciences, University of Defence,  
Hradec Kralove, Czech Republic**BACKGROUND-AIM**

Varicella is a highly infectious, disease, which is, however, preventable and an effective vaccine is available. The aim of this study was to provide complex data about the incidence of VZV infection in a large cohort using tools of molecular epidemiology.

**METHODS**

Samples from VZV lesion from both in- and outpatient patients (from Faculty Hospital Hradec Králové) were analyzed as part of our molecular genetic study of varicella-zoster virus during years 2009 – 2016. The VZV genotyping was performed using both SNP analysis and, in select samples, whole genome sequencing.

**RESULTS**

Altogether, 463 persons with varicella were younger than 18 years and 160 persons (25,7 %) were older. A total of 18 patients (2,9%) were immunocompromised. Fifty patients reported the second incidence and 1 patient even third incidence of varicella. Patients reported with varicella during the entire calendar year with two peaks in incidence - in winter (January, February) and late spring (May and June). This is contrary to the data repeatedly reported in literature, which show this disease being seasonal in the temperate climate countries with a peak during winter months. While chickenpox is typically childhood disease in our country, we also report a detailed analysis of 160 adults..

Genotypic analysis confirmed our previous findings and showed a higher prevalence of the European VZV wild type strains 2 (E2 - 65,6%) compared to European VZV wild type strains 1 (E1 - 33,7%). In one of the VZV isolates (from an immunosuppressed girl with uncomplicated varicella) we describe newly found mutations at positions 33924 and 95262.

**CONCLUSIONS**

Our study has shown a continuous incidence of chickenpox throughout the entire year and confirmed predominance of the E2 VZV strain.

This work was supported by the Organization development plan 1011.



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