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Acute respiratory infections with an epidemic character have been recognised since antiquity and remain of major global public health importance today. Despite much greater understanding of the nature and diversity of respiratory viral agents, diagnosis and control of these human infections remain as challenging to health systems as ever. Approximately 40% of mortality due to lower respiratory tract infections (over 4 million deaths) is attributable to respiratory viral infections every year. Influenza and RSV are the major predictable contributors to this, but the unexpected emergence and spread of high morbidity viruses such as SARS and MERS, transmissible through respiratory routes, is a reminder of the potential for new and emerging respiratory viruses, including pandemic influenza, to cause havoc in societies. The recent upsurge in enteroviral D68 viruses which have a respiratory tropism, but are also associated with acute flaccid paralysis, may be an example of changing ecological niche and a reminder that diagnostic detection is a key parameter for any control measure, including development of vaccination strategies.

Is the goal of a universal influenza vaccine realistic or would better population control of influenza be more achievable by a bespoke age segmented approach? Outcomes of the adoption of newer influenza vaccines and some of the more recent issues associated with vaccine strain selection for both live attenuated and subunit vaccines are discussed.

There is much to gain from a more systematic approach to tracking respiratory virus evolution across the globe. The impact of viral diversity is not well understood for RSV, despite intensive study over decades, and this is also true for many other respiratory viruses. The experience of creating a tracking system to enable influenza vaccine strain selection informs the development of a global surveillance programme for RSV, to support the licensure of interventions. New and faster methods for detection and molecular epidemiology of respiratory viruses will help to fill knowledge gaps.
The hepatitis A virus (HAV) is the prototype of genus *Hepatovirus*, within the *Picornaviridae* family, although genomic, structural, antigenic and biological properties make HAV a unique virus. Its transmission is mostly through the faecal-oral route although parenteral spread may also occur. In developed countries, the men-having-sex-with-men (MSM) group is particularly prone to suffer large outbreaks and foodborne outbreaks still frequently occur due to the global food trade.

HAV has an extremely stable capsid, a feature required for its biological cycle that may be related to its very special codon usage, shaped by fine-tuning translation selection and resulting in a highly regulated ribosome traffic pace. The requirement of stability for a virus with long periods in the environment, out of the host body, is ensured by negative selection of mutations affecting capsid rare codons mainly encoding surface residues located in the epitope sites hampering the emergence of new serotypes. Antigenic variants, however, may arise in particular situations of immune pressure, although HAV codon usage may be a genomic constraint to antigenic variability. Additionally, the deviated codon usage of hepatoviruses compared with that of their hosts may suggest the occurrence of a virus ancestor with an optimized codon usage with respect to an unknown ancient host.

In an MSM outbreak investigation, HAV evolution at the quasispecies level, in non-vaccinated and vaccinated patients, revealed higher diversity in epitope-coding regions of the vaccinated group. Although amino acid replacements occurring in and around the epitopes were observed in both groups, their abundance was significantly higher in the quasispecies of vaccinated patients, indicating ongoing processes of fixation. Our data suggest positive selection of antigenic variants in some vaccinated patients, raising concern for vaccination polices directed to the MSM group.
In 2014, 2016 and 2018 the U.S. experienced a dramatic increase in cases of acute flaccid myelitis (AFM). Similar cases were reported worldwide including many European countries. AFM is defined by the acute onset of focal limb weakness associated with MRI evidence of a multi-segmental predominantly grey matter lesion in the spinal cord. In 2018 there were 235+ cases from 41 states in the U.S. confirmed by the CDC. AFM patients are predominantly children (90%), present in the summer and fall, and have a viral-like prodrome that precedes onset of neurological disease. The temporal and geographic association of AFM with a parallel emergence of EV-D68-associated respiratory infections led to the suggestion that EV-D68 was the major causal agent of AFM, a supposition that now has convincing epidemiological support. The clinical and laboratory features of AFM will be discussed, along with new evidence finding anti-EV and anti-EV-D68 specific CSF antibody responses in AFM cases, providing the strongest causal link to date between EV-D68 and AFM. EV-D68 strains can infect neuronal cell cultures and have been shown to be preferentially transported via retrograde as opposed to anterograde axonal transport. Strains differ in their receptor utilization in neurons, and this may contribute to neurotropism. Mouse models of EV-D68 AFM developed by our laboratory and others clearly show infection of and loss of motor neurons corresponding to weakness. The use of these models to understand pathogenesis and to evaluate potential therapeutic agents including antivirals, immunotherapy, and vaccine strategies will be reviewed.
In recent years, it has emerged that mutations in genes encoding proteins of innate or cell-intrinsic immunity may underlie rare primary immunodeficiencies and predispose to severe infections in humans. We have previously demonstrated a mutation in the transcription factor interferon (IFN) regulatory factor (IRF)3 causing impaired antiviral IFN responses in a patient with severe herpes simplex encephalitis. In most individuals, varicella zoster virus (VZV) causes varicella upon primary infection and zoster during reactivation. However, in a subset of individuals, VZV may cause severe disease, including infection in the central nervous system (CNS). Recent studies by our group have demonstrated that defects in the immunological DNA sensor RNA polymerase III (POL III) confers impaired antiviral interferon responses and selective increased susceptibility to VZV infection, thus providing fundamental new insight into VZV immunity. Here I will present our data on the identification of functionally defective genetic variants in different subunits of POL III in children as well as in adults causing disease manifestations, including encephalitis, vasculitis, and stroke. The roles of POL III in cellular housekeeping and immune surveillance during VZV infection are described and the latest knowledge on POL III and DNA sensing in VZV infection are discussed. In addition, I will highlight emerging outstanding questions related to POL III in immunity to VZV and other alpha-herpes viruses, and how this new insight may be translated into clinical medicine. Finally, I will present new unpublished results linking defects in cellular autophagy pathways to recurrent viral (Mollaret) meningitis in humans.
Noroviruses are the leading cause of epidemic and sporadic acute gastroenteritis in humans worldwide and the most common cause of foodborne illness in the U.S. Although TaqMan-based RT-PCR assays continue to serve as robust methods for the sensitive detection of noroviruses, new technologies such as next generation sequencing and digital PCR have become available and have great potential for detection and typing of these viruses including in environmental samples. Since 2009, norovirus outbreaks in the United States are reported to a national norovirus outbreak surveillance network (CaliciNet) and several other networks capture the rate of endemic disease in hospitalized and outpatient children with AGE with the rate in healthy controls. These surveillance networks capture norovirus genotype distribution in young children and are able to detect newly emerging strains in realtime. Since 2002, new GII.4 variants have emerged every 2-3 years and caused the majority of norovirus infections globally. In November 2015, a new GII.4 virus emerged which had more than 2% nucleotide difference with the GII.Pe-GII.4 Sydney viruses that circulated since 2012. Complete genome sequencing showed that this strain was a recombinant GII.4 Sydney capsid type with a GII.P16 polymerase type (GII.4 Sydney[P16]). ORF1 sequences including the GII.P16 polymerase gene has been associated with additional genotypes including GII.2, GII.3, and GII.13 whereas GII.Pe was primarily associated with GII.4 Sydney. These results indicate that both emerging of new GII.4 capsid variants as well as ORF1-ORF2 recombinants have become major drivers in the epidemiology of norovirus and that dual typing of norovirus strains should become standard for strain surveillance in both clinical and environmental samples.

Despite numerous attempts, in vitro culture of human norovirus has been unsuccessful until recently when successful replication of human norovirus in non-transformed human intestinal enteroids was reported. Successful implementation of this novel culture method in a few laboratories will allow answering many important questions of the epidemiology, pathogenesis and effectiveness of control measures. This major breakthrough in the field will also help answering important questions for the development of norovirus vaccine candidates several of which are in clinical trials. However, several significant challenges of creating a robust norovirus vaccine remain including if broad protection against multiple genotypes can be obtained, and for how long such a vaccine would protect against future symptomatic infections. Therefore, global surveillance networks of both outbreaks as well as pediatric infections, where the burden is highest, will be critically important to monitor emergence of new viruses and changes in genotype distribution.
NOVEL STRATEGIES IN VACCINE DESIGN IN RESPONSE TO EMERGING VIRAL THREATS

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There is an increasing emergence of new viral threats, like Zika, West Nile, MERS, Ebola with the risk of repeated outbreaks, epidemic or pandemic spread e.g. with shifted influenza A, mutated neglected zoonotic and vector-borne virus or unknown virus. Therefore, there is a need for novel strategies in vaccine design and plans for their rational use. One goal is to establish a few fast, flexible and universal vaccine platforms. Critical factors are response-time, production-speed and capacity, induction of cellular immunity to protect against disease in addition to protective antibodies to protect against infection and safety of the new vaccines.

Second generation DNA vaccines may be one such platform: New generation of plasmid DNA (or self-replicating RNA) vaccines have gained potency in humans by using multiple, rationally selected genes, optimizing gene- and vector design and optimizing delivery, but they may still require multiple shorts for some virus diseases. A more potent one-shot strategy are DNA vaccine delivery using recombinant replication-competent virus, like rVSV, rAd26 and rPox that, however, may have safety issues and immunity to the delivery virus which prevent their reuse in boostings or their use for other infections.

Results from a broad protective influenza plasmid vaccine design and lesson learned from the successful efficient Ebola rVSV-ZEBOV-GP ring-vaccinations during the ongoing DRC outbreak will be discussed in this context.

The last 20 years has seen the emergence of several new virus threats (SARS, MERS, Hendra, Nipah) and devastating outbreaks of re-emerging viruses (Ebola, influenza, West Nile). This ever-present threat of emerging and re-emerging viruses with epidemic and pandemic potential necessitates novel vaccine strategies that are rapidly available, safe, and effective in eliminating spread amongst those at risk. Critical factors to consider include rapid rational design, production speed and capacity, safety, stability, and induction of cellular and humoral immunity that protect against both virus acquisition (prophylactic) and disease (therapeutic). Second generation DNA vaccines may be one such platform: New generation of plasmid DNA (or self-replicating RNA) vaccines have gained potency in humans by using multiple-rationally selected genes, optimizing gene- and vector design and especially delivery, but may still require multiple immunizations for some virus diseases. A more potent single-immunization strategy are DNA vaccine delivery using recombinant replication-competent virus, like rVSV, rAd26 and rPox that, however, may have safety concerns and immunity to the delivery virus which prevent their reuse in boostings or use for other infections.

Results from a broad protective influenza plasmid vaccine design and lesson learned from the successful efficient Ebola rVSV-ZEBOV-GP ring-vaccinations during the ongoing DRC outbreak will be discussed in this context.
Cytomegalovirus (CMV) infection remains a frequent cause of morbidity and mortality in the transplantation setting. The use of antivirals with relatively high intrinsic activity against CMV, such as (val)ganciclovir or foscarnet, administered either prophylactically or pre-emptively, has dramatically decreased the incidence of early CMV disease (occurring within the first 100 days after transplant). Nevertheless, overtreatment leading to drug-related toxicity and occurrence of late CMV disease continue to be non-negligible drawbacks of both preventative strategies. Functional CMV-specific T cells are essential in the prevention and resolution of CMV viremia and disease. Combined kinetic analyses of plasma CMV DNA load and routine monitoring of CMV-specific T-cell responses, which may easily conduct nowadays by means of commercially-available interferon-gamma-release assays (Quantiferon CMV or ELISPOT) or by flow cytometry (ICS), may help to individualize (optimize) antiviral prophylaxis and preemptive antiviral therapies. Different strategies of clinical intervention based upon routine monitoring of CMV-specific T-cell immunity in both allogeneic hematopoietic stem cell transplant recipients and solid organ transplant recipients will be discussed.
INFLUENZA VIRUS LABORATORY SURVEILLANCE IN A CLINICAL HOSPITAL, SARAJEVO, BOSNIA & HERZEGOVINA

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Aim: “To estimate the influenza (Inf) virus laboratory surveillance in a clinical hospital setting in Sarajevo, Bosnia and Herzegovina (B&H), entity of Federation of B&H (FB&H), by calculation the total time/resources spent by laboratory per week analyzing the suspected specimens and reporting the influenza results to the surveillance system.”

Methods: “Laboratory calculation data based on real-time RT-PCR and virus isolation methods used for processing of one to fifteen samples were available from the reference laboratory (RL) at the Clinical Center of the University of Sarajevo, Unit for Clinical Microbiology during the flu season.”

Results: “Average weekly time for initial steps involving reception of samples in the laboratory, recording, aliquoting and sample archiving, followed by primary diagnostic (screening laboratory test) based on typing of InfA and InfB, and recording the results to the laboratory database was 3 hours and 15 minutes. Secondary diagnostic including subtyping of InfA (pdm InfA, pdm InfA/H1 and InfA/H3) and InfB (InfB/Vic and InfB/Yam) by real-time RT-PCR, analysis and interpretation of the results, recording the results to the laboratory database, and aggregation and reporting the data, required on average 2 hours and 29 minutes per week. For virus isolation (cell culture, hemagglutination) and recording the results to the laboratory database, laboratory on average spent 7 hours and 22 minutes.

Conclusions: “Total estimation on average time that influenza virus laboratory surveillance takes per week was 13 hours and 6 minutes, which is almost 35% of entire working time of the laboratory and its staff during the flu season.”

Keywords (MESH): Influenza, Human; Bosnia and Herzegovina; Real-Time Polymerase Chain Reaction; Cell Culture Techniques;
**[O001] INFLUENZA VIRAL LOAD MEASUREMENT IN RESPIRATORY SAMPLES FROM PATIENTS WITH SEVERE RESPIRATORY SYMPTOMS AS A MARKER OF PROGNOSIS**

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**Aim:** The link between influenza virus (IV) viral load (VL) in respiratory samples and disease severity is not clearly established. This study was designed to assess IV-VL in respiratory samples from flu patients admitted to intensive care unit (ICU).

**Method:** Patients admitted to the ICU for IV infection, as documented by RT-PCR, with respiratory failure were included in the study during the last 5 flu-seasons (2014-2018). Routine ICU management parameters were recorded. Real-time amplification Ct values for IV and cell GAPDH gene were measured in each respiratory sample collected at ICU admission.

**Results:** Among 105 included patients, 59 (56.1%) presented an acute respiratory distress syndrome (ARDS). The overall mortality was 21%. IV load assessed by amplification Ct-values and virus-over-cell ratio (expressed as log₁₀) in each respiratory sample ranged from 20 to 40 and -5.2 to 3.7, respectively, and did not differ according to the type of sample and IV-A or -B type. Cell richness was higher in samples from ARDS patients compared to non-ARDS (p=0.0003) but no difference was noted for IV Ct-values. In ARDS-patients, IV Ct-values (p=0.020) and the virus-per-cell ratio (p=0.038) were significantly higher in sample from patients who eventually died compared to those who survived. These 2 parameters remain independently associated with mortality with an odd-ratio of 1.21 and 2.19, respectively (p<0.05).

**Conclusions:** While IV-VL does not seem to predict disease evolution in ICU flu-patients, standardized measurement of IV-VL in respiratory samples could be useful in ARDS patients to identify patients at higher risk of mortality.
MOLECULAR EPIDEMIOLOGY OF RHINOVIRUSES/ENTEROVIRUSES AND THEIR ROLE ON CAUSE SEVERE AND PROLONGED INFECTIONS AMONG HOSPITALIZED PATIENTS.

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Aim: Rhinovirus (HRV) and Enterovirus (EV) are two of the most common respiratory viruses, causing from mild to severe respiratory syndromes (e.g. pneumonia). Here we report the molecular characterization of HRV/EV strains detected in a hospitalized-based population.

Method: From September 2017 to December 2018, respiratory samples collected from upper* or lower** respiratory tract were tested for respiratory viruses using a set of laboratory developed multiplex real-time assays. All samples positive for HRV/EV were typed by sequencing the VP4-VP2 region.

Results: A total of 201 (8.0% of the total) patients were positive for a HRV infection. Median age of HRV-positive patients was 9 years (range 10 days-96 yrs). In 41 cases (20.4%) HRV was detected in co-infection with other respiratory viruses. Peak of cases were observed in the Sep-Nov period of 2017 and 2018. HRV-A was detected in 118/201 (58.7%) patients, HRV B in 18 (9.0%), HRV-C in 39 (19.4%) and EV in 13 (6.5%), while no typing results was obtained for 13 (6.5%) cases. A total of 10 patients were hospitalized in intensive care unit and in 12 immunocompromised patients a prolonged infection (>30 days) was observed (median duration 62 days, range 17-316). Based on typing and temporal analysis, 3 HRV outbreaks were identified, mainly in neonatal and oncoematology wards, caused by HRV-A89, A49, C43.

Conclusions: Clinical impact of HRV/EV infections is not limited only to a common cold but these viruses should be considered as significant respiratory pathogens.

*FLOQSwabs™ collected in UTM
**bronchoalveolar lavages
Aim: To identify mutations in human RSV associated with severe disease by whole genome sequencing (WGS) in children <2 years of age.

Method: HRSV strains from severe and non-severe cases based on a clinical disease severity score were identified in three Catalan paediatric hospitals and four primary care centers. WGS was performed on Illumina MiSeq platform in order to compare their genetic features.

Results: From October/2015 to May/2018, we analyzed 58 HRSV-A and 40 RSV-B strains. Of HRSV-A strains, 31 strains belonged to infants with severe disease and 27 non-severe cases. Of those with HRSV-B, 15 belonged to infants with severe infection and 25 to non-severe. For HRSV-A, 372 nucleotide mutations were common to both groups, 664 were exclusively identified in non-severe cases and 967 in severe cases. GWAS study revealed that 15 mutations were statistically significant, of which 4 (3 non-synonymous) were associated with severe disease: E262K in G protein and R773H, A1617V and 4863G>A in L protein. For HRSV-B, 542 nucleotide mutations were identified in both
groups, 975 in non-severe cases and 1492 in severe cases. Nine mutations were statistically significant, of which just one synonymous mutation was associated to severe cases: 3129C>T in the L protein.

**Conclusions:** We identified specific mutations in HRSV strains from children with non-severe and with severe RSV disease. Identification of viral genetic markers by GWAS could help to predict clinical outcomes and represents a step-forward to personalised medicine. Nevertheless, validation in further studies including a larger sample size is warranted.
SEVERE RSV INFECTION IN EARLY CHILDHOOD AND RISK OF ASTHMA LATER IN CHILDHOOD

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Aim: Respiratory syncytial virus infection (RSV) during infancy has been associated with asthma later in life. We explored the risk of asthma hospitalization after early childhood RSV hospitalization.

Method: We performed a retrospective cohort analysis using Danish National hospitalization discharge registers. Children born between January 1995 and October 2012 and admitted to hospital within the first 12 months of life with a diagnosis of RSV-infection (ICD10: J121, J205, J210, B974), were compared to children with urinary tract infections (UTI; N390, P393), and those with non-RSV acute respiratory infection (ARI; J00-J05, J09-J17, J20-21). Children with prior asthma or wheezing were excluded. All children were followed-up until diagnosis of asthma (J45-J46) or wheeze (R062, J209), death, or age 6, and incidence rate ratio’s (RR) were calculated.

Results: 21504 children with RSV, 23985 with non-RSV RTI, and 1437 with UTI hospitalizations were included. The incidence of asthma or wheeze was 36.0 per 1000-person years after RSV hospitalization, 12.0 after UTI, and 17.3 after non-RSV ARI, respectively. The RR of RSV versus UTI was 2.99 (95% confidence interval (CI): 2.44-3.68), 2.08 (95% CI: 1.97-2.19) for RSV versus non-RSV ARI, and 1.44 (95% CI: 1.17-1.77 for non-RSV ARI versus UTI.

Conclusions: Early childhood RSV hospitalization increases the risk of subsequent hospitalization with asthma or wheeze. Albeit this study-design precludes establishing a causal link, our findings suggest RSV-vaccinations have the potential to significantly reduce disease burden due to asthma.

Acknowledgements: This project was funded by the Innovative Medicines Initiative 2 Joint Undertaking (grant No 116019).
Aim: To prevent nosocomial influenza virus infections in our tertiary academic hospital with limited isolation capacity, influenza suspected patients are rapidly screened using the assay*. The efficacy of patient isolation based on rapid influenza diagnostics during influenza season 2017/2018 was evaluated and optimized for the next season.

Method: We first evaluated the timeline between patient arrival at the Emergency Department and availability of laboratory results for both rapid and conventional RT-PCR. Secondly, to investigate nosocomial spread, samples from 101 influenza B virus positive patients and from 7 influenza A(H3N2) virus positive patients and HCWs from one ward were Sanger sequenced.

Results: Between December 2017 and May 2018, 408 Xpress Flu/RSV assays were performed of which 157 (38%) tested influenza positive. The median time between sampling and result was <2hr, while this was >24hr for the conventional RT-PCR. Influenza B sequence analysis demonstrated ten patient clusters of which three had unique genotypes compared to (inter)national data. Epidemiological analysis identified five isolated nosocomial infections but did not support nosocomial transmission within the clusters. In contrast, the A(H3N2) viruses had the same unique genotype, confirming epidemiologically suspected nosocomial transmission.

Conclusions: Rapid diagnostics for influenza virus substantially reduced turn-around time, allowing efficient patient isolation. Epidemiological and cluster analysis yielded only five (5%) nosocomial influenza B virus infections together with an influenza A(H3N2) cluster containing patients as well as unvaccinated HCWs. These findings illustrate the need for higher influenza vaccination coverage of HCWs.

*Xpert® Xpress Flu/RSV
Aim: Steady increase in HIV drug resistance (HIVDR) has been demonstrated in individuals initiating first-line antiretroviral therapy (ART) globally. We present the results of a pilot HIVDR surveillance system which assessed feasibility for HIV transmitted DR (TDR) surveillance implementation in the EU.

Method: Nationally appointed experts from EU/EEA countries were invited to participate in the pilot study. Countries submitted data on individuals newly diagnosed with HIV in 2015 who were tested for antiviral susceptibility prior to ART - either as case-based (including demographic, diagnosis, clinical and resistance variables) or aggregate data by sex, transmission route and drug class. For interpretation of genetic sequences into susceptible, (potential) low, intermediate or high resistance level, the Stanford HIV database algorithm was used.

Results: Nine EU countries participated successfully in the pilot, with six reporting 1680 case-based data and four reporting aggregated data on 1401 cases. Two-thirds of countries linked the laboratory and epidemiological data. The completeness of reporting ranged from 21% for integrase sequences...
to 100% for sex and prior ART. Sequence data were available for 1,417 cases and overall 14.7% (CI: 12.9 – 16.3%) of patients showed TDR to at least one ARV drug with 0 to 18% variation between countries. Highest TDR prevalence was observed to non-nucleoside reverse-transcriptase inhibitors (NNRTIs) with 8.6%, followed by 5.1% to NRTIs and 2.0% to protease inhibitors.

Conclusions: Standard HIVDR data reporting was feasible for the nine participating countries. Due to legal barriers for case-based data sharing, an aggregated EU-wide HIV-TDR surveillance system will be further developed.
HPV 16 SEROPOSITIVE HEALTHY MEN ARE SIGNIFICANTLY YOUNGER THAN SERONEGATIVE ONES

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Aim: HPV 16 is the commonest and the most oncogenic of over 200 known human Papillomavirus (HPV) genotypes. Data on humoral immune response to HPV infections among healthy individuals are scarce. In this study we characterize the seroprevalence of HPV 16 infection among healthy blood-donating men.

Method: Sera of 124 healthy non-vaccinated men (blood donors) were analysed for the presence of antibodies against HPV 16 L1 capsid protein using VLP-based ELISA assay*. Results were also compared with HPV 16 antibody status of HIV positive men-who-have-sex-with-men (MSM).

Results: Mean age (SD) of the study participants was 46.7 (11.2), age range from 20 to 66 years. A total of 36 individuals – 29% (95% CI (21.4-37.2)) – were above the calculated cut off for positivity (0.22). Mean (SD) corrected optical density (OD) was 0.52 (0.91). The mean ages (SD) of seropositive and seronegative donors were 42.4 (12.5) and 48.4 (10.2), respectively (p_anova =0.006). Although HIV positive MSM are known for the high prevalence of HPV infections, the seroprevalence among these two populations was similar (29% versus 31%, respectively).

Conclusions: Our study showed that a third of blood donating healthy men had a positive antibody titer against HPV 16. Higher seroprevalence at a younger age may mean higher risk of HPV acquisition at this age, but lack of persistence of the antibody to a later age.

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Aim: This prospective study was done to determine the incidence of Cytomegalovirus (CMV) reactivation and its impact on mortality and length of stay (LOS) in Intensive Care Unit (ICU) among critically ill sero-positive cirrhotic adults.

Method: Sero-positive (anti CMV IgG-positive) non-immunosuppressed critically ill cirrhotic adults in Liver-ICU (>72 hours) were assessed for CMV reactivation with CMV-plasma-DNAemia ≥500 (2.69 log_{10}) IU/ml by quantitative real-time polymerase chain reaction (day 0, 7, 14, 21), excluding patients with prior CMV reactivation (on day 0).

Results: Of 84 enrolled Liver-ICU patients [73 men, median age: 49.5 years, interquartile range (IQR): 40-57.5], 166 blood samples were tested. Cumulative incidence of CMV reactivation was 30.9% (95% confidence interval, CI: 19.1- 44.80) at 7-day follow-up. The incidence rate/density of CMV reactivation was 2.75% per person-day (95% CI: 1.68 - 4.26% person-day) during 21-day follow-up. Patients with CMV reactivation frequently presented with Acute-on-chronic liver failure (63.16%; p=0.003). Significant factors for CMV reactivation were bacterial infections (p=0.031, Odds Ratio (OR):0.07, 95% CI: 0.01- 0.78) and leucocytosis (p=0.047, OR: 1.15, 95% CI: 1.00-1.32). ICU-Mortality did not differ between patients with and without CMV reactivation (90% vs. 88.57%, p=1.00). CMV reactivation was not independently associated with time to death [Hazard Ratio (HR): 1.18, 95% CI: 0.65 to 2.15, p=0.58] and ICU-LOS (9.50, IQR 8-16.50 vs. 12, IQR 8-18 days; HR: 1.12; 95% CI: 0.64-1.97, p=0.68).

Conclusions: Though CMV reactivation was frequent among critically ill, non-immunosuppressed cirrhotic adults, it was not significantly associated with increased mortality and prolonged LOS in Liver-ICU.
Aim: Public Health England (PHE) and NHS England (NHSE) plan to use historical laboratory data to re-engage persons with a positive Hepatitis C virus (HCV) antibody result for PCR testing and treatment if required. The aim of this project was to evaluate the utility of this data.

Method: Antibody and PCR data from 1996-2019 was collected from several regional laboratories within the West Midlands, UK. This was cross-referenced against a list of historical antibody results collected by a laboratory PHE Second Generation Surveillance System (SGSS).

Results: From the SGSS list of the laboratories where data was collected (N=1491), PCR data was found for 988 (66%). Of these 276 (28%) had received treatment. Of the 712 (72%) persons untreated, 347 (49%) were PCR negative and 365 (51%) were PCR positive. In an analysis of all PCR data collected 3738 identifiable patients), 1284 had evidence of presumed treatment or clearance (a detectable to undetectable RNA result). The mean time to negative PCR from the first positive was 784 days (see Figure 1).

Conclusions: A third of patients with a positive HCV antibody test had no follow-up and highlight a group to actively re-engage. Patients with detectable HCV RNA become undetectable on average just over 2 years after their first positive PCR result. This suggests the HCV pathway is not sufficiently timely and hence these individuals would remain sources of viral transmission. Historical laboratory data has limitations, however can direct HCV treatment strategies.
Figure 1 - Time to first negative HCV RNA result
PROGNOSTIC ROLE OF LIVER FUNCTION TESTS IN PEDIATRIC DENGUE ILLNESS

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Aim: “Liver involvement in dengue illness is common and can leads to acute liver failure (ALF). No single method can effectively identify patients at risk for disease progression and bad outcome. We aimed to determine liver dysfunction, kinetics of liver function tests (LFT) and severity of hepatitis on outcome in pediatric dengue illness”

Method: We enrolled hospitalized children (1-12 years) with dengue infection (July2014-July 2015). Serial monitoring of LFT was done in confirmed dengue cases. Patients classified into non-severe (NSD) and severe dengue (SD). Severity of hepatitis graded: mild, moderate and severe hepatitis”

Results: “A total of 102 children (66, boys), median age 72 (12-144) months analyzed (NSD, n=41; SD, n=61). Elevated transaminases (92%) were the most common abnormality; aspartate (AST) and alanine transaminase (ALT) in 87% and 82% respectively. Maximum abnormalities in LFT peaked at day 5 (AST, ALT) and day 7 (ALP, GGT) of illness. Elevated transaminases higher in SD than NSD (100% vs. 80%, p=0.006). Severe hepatitis group developed organ dysfunction like altered sensorium (p<0.001), ALF (p<0.001), acute kidney injury (p<0.001) and shock (p<0.001) more commonly than mild to moderate hepatitis. Fourteen patients died, 2/3rd from severe hepatitis group (p<0.001). On binary logistic regression presence of severe hepatitis and shock at presentation was independent predictor for occurrence ALF (OR: 77; 95%CI: 13-457, p<0.001) and mortality (OR: 55; 95%CI: 4.6-66, p<0.001) respectively”

Conclusions: “Remarkable number of children with dengue have liver involvement. Severe hepatitis in dengue associated with significant organ dysfunction and poorer outcome”
Aim: In developed nations the prevalent hepatitis E virus (HEV) strains are genotypes 3 and 4, that are primarily zoonotically transmitted through the consumption of animal products. The primary species that are considered reservoirs for transmission to humans are swine and wild boar and the professional exposure to the reservoirs is considered a risk factor for human infection. The aim of this study is to evaluate the epidemiology of HEV in humans and wild boar in the Park of the Ticino, Italy.

Method: in the period 2016-2017, 3008 wild boars’ serum were tested to evaluate the presence of HEV IgG and 1156 wild boars liver fragments were analyzed to evaluate the presence of HEV-RNA and genotyped. Moreover, in the same period, 40 serum samples of healthy forestry workers in the Park of the Ticino and 120 blood samples from donors, as control group, were analysed to evaluate the presence of HEV IgM and IgG.

Results: The study revealed: i) 8.7% prevalence of IgG against HEV for wild boars; ii) 3.7% positivity of HEV-RNA in wild boar liver samples, all the strains belonging to genotype 3; iii) 10.0% prevalence of IgG against HEV for forestry workers; iv) 1.7% prevalence of IgG against HEV for blood donors.

Conclusions: The different prevalence of HEV IgG between the forestry workers (10.0%) and the blood donors (1.7%) reveals a statistically significant difference between the two groups (p=0.0347), highlighting that the exposure workers categories had a greater risk factor, 6.5 times higher, than the control group.
[O012] EPIDEMIOLOGICAL SURVEILLANCE AND MOLECULAR EPIDEMIOLOGY OF ASEPTIC MENINGITIS IN QATAR

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Aim: “This study aims at characterizing the etiological agents of aseptic meningitis (AM) in Qatar”

Method: “A retrospective analysis of suspected AM cases was performed between September 2017 and September 2018, followed by molecular characterization and genotyping of circulating human enteroviruses. HEV genotyping was carried out by sequencing the complete and partial genes encoding the VP1 and VP4/VP2 capsid proteins, respectively”

Results: “About eight percent of all admitted meningitis cases were due to viral infection. Interestingly, 65.7% of reported cases were males, compared to 34.2% in females (P=0.024). Moreover, data showed that infants (less than one year) (54.5%) and adults (10-17 years old) (20.8%) are the most frequently AM affected groups. We observed no specific seasonality of AM agents, but slight rise was recorded during summer season (May- July). Human enterovirus was identified as the most causative agent of AM in our study, accounting 58.4% of all cases. Molecular characterization was successful in only 42.5% (n= 20) of the patients, with the identification of serotypes echovirus 3 (n= 15, 75%), echovirus 11 (n=4, 20%) belonging to human genera B and the serotype EV-C105 (n=1, 5%) belonging to human genera C. Infections were approximately equally divided between expatriates (N=56, 53.9%) and Qataris (n=48, 46.1%)”

Conclusions: “Our data showed that enterovirus are responsible for more than 58% of AM cases in Qatar. Molecular analysis revealed the circulation of emerging strains such as EV-C105, which has been associated with flaccid paralysis in other parts of the world”
[0013] CIRCULATION OF NON-POLIO ENTEROVIRUSES IN 24 EU/EEA COUNTRIES BETWEEN 2015 AND 2017


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Aim: Enteroviruses (EVs) can cause severe infections, especially in young children. Non-polio EV infections are not notifiable in most European Union (EU)/European Economic Area (EEA) region countries, and surveillance varies largely between these countries. We collected and analysed the available EV data across EU/EEA countries to assess the current epidemiological situation and capacities for surveillance.

Method: Aggregated data for 2015-2017 on any EVs detected were requested from all EU/EEA countries through national enterovirus reference laboratories. Information included EV types detected by month, patient age group, symptoms and specimen type. Sequence data on capsid region of three selected EV types were collected.

Results: Respondents from 24 countries participated; typing for 70% of specimens (11,608/17,136) revealed 66 EV types. Children under 5 years of age accounted for 66% of EV infections, and 47% of cases had neurological symptoms, 0.5% were reported with myocarditis and 1% died. Coxsackievirus A6 (CAV6) was the most frequently detected type (n=1,665) with 65% of infections linked to hand, foot and mouth disease, followed by echovirus 30 (n=1,412) associated with neurological symptoms (82%). Sequences available from 18 countries showed circulation of newly emerging strains of EV-A71, CAV6 and EV-D68 in the region.

Conclusions: This is the largest investigation of EVs circulation in EU/EEA region and confirms the existence of EV surveillance activities in EU/EEA Member States. It demonstrates the wide circulation of EVs associated with neurological symptoms in the region. Collaborations between the Member States could be encouraged to harmonise non-polio EV surveillance further at national level.
Aim: The sensitivity of enterovirus (EV) and human parechovirus (HPeV) detection assays is critical for their effectiveness in virus diagnostics. The performance and target range of typing methods is similarly important for disease monitoring and surveillance for emerging EV types, such as EV-D68. However, there is little systematic information on absolute sensitivity of assays currently used in clinical virology or public health laboratories. This was evaluated by blinded testing of a RNA transcript panel representing the different EV species and HPeV.

Method: 38 clinical and public health laboratories, contacted primarily through ENPEN, participated in the study. Each was provided with blinded full genome length RNA transcripts samples representing EV species A (EV-A71), B (E30), C (CAV21) and D (EV-D68) and HPeV-3 at defined molecular concentrations (10 or 1000 RNA copies/5µl) and a human rhinovirus species C. Results were reported prior to decoding.

Results: Samples were tested in 62 screening (46 in-house, 16 commercial) and 38 typing assays. Detection assays performed consistently with 96%-100% detection frequencies of 1000 copy samples and 94.9% specificity. Lower detection frequencies of the 10 copy samples were observed (in-house: 80% and commercial assays: 57% mean detection frequency) with several commercial assays failing to detect multiple EV species. Typing assays were highly specific (100% correct virus identifications) and sensitive (84%).

Conclusions: Assay sensitivity for EV and HPeV detection is important as viral loads in CSF are often low. However, a substantial proportion of commercial assays failed to detect 10 copy transcript controls.
Aim: “To determine the value of serology and PCR for the diagnosis of tick-borne encephalitis”

Method: “Review of data on tick-borne encephalitis virus (TBEV) serology and real-time PCR from our laboratory information system since 2007.”

Results: “Between January 2007 and December 2018, 3453 serum samples were tested for TBEV IgM and IgG by ELISA. For 3014 of the serum samples, a paired cerebrospinal fluid (CSF) sample was also available and tested for TBEV IgG. During these 12 years, 82 cases of acute TBEV infection were identified by IgG and IgM ELISA tests (median 6 per year, range 3-14). CSF for analysis of intrathecal antibody synthesis was available in 68 of these patients; 37 (54 %) had an elevated antibody index, suggesting TBEV-specific intrathecal antibody synthesis. CSF for real-time PCR testing was available from 37 patients. None of them had detectable TBEV RNA in CSF. On the other hand, TBEV RNA was detected in 6 CSF samples from three patients with severe encephalitis who did not have TBEV antibodies in serum. All three patients had been treated with the B cell-depleting antibody Rituximab before the encephalitis developed.”

Conclusions: “In “normal”, immunocompetent patients, diagnosis of TBEV infection relies exclusively on serology, and PCR detection of TBEV RNA has no additional value. However, if patients have been treated with B cell depleting antibodies like Rituximab, detection of TBEV RNA in CSF (and maybe also in other specimens like urine) is the only way to make a diagnosis of tick-borne encephalitis.”
[O016] DISSECTION OF THE HCMV-SPECIFIC ANTIBODY RESPONSES IN LUNG-TRANSPLANT RECIPIENTS

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Aim: HCMV is an important pathogen in lung-transplant recipients (LTRs). The risk for HCMV replication depends on the donor’s (D) and recipient’s (R) serostatus as well as on the elicitation of HCMV-specific immune responses. In this study we analyzed the glycoprotein B (gB) and pentameric complex (PC)-specific IgG antibody (AB)-responses, as well as the AB-mediated NK-cell functions following lung transplantation.

Method: 35 R+ and 26 D+/R– LTRs were included in the study. From each patient, follow-up plasma samples were collected in a three-month interval in a one (R+) or two (D+/R–) year period post-transplantation. 14 R+ and 9 D+/R– patients showed a viremic episode >1000 copies HCMV DNA/mL in the follow-up. PC-specific and gB-specific IgG titers were determined by ELISA. The NK-cell response was determined using NK92-CD16a cells in a serum-dependent focal expansion assay and a CD107 cytotoxicity assay.

Results: The R+ LTRs reached a higher level of gB-but not of PC-specific IgG1-titers in the first year post-transplantation compared to healthy seropositive individuals (p<0.0001). Functional analysis of gB-specific ABs exhibited that they lead to significantly better NK-cell activation in R+ LTRs than in healthy HCMV-seropositives (p<0.0001), shown by higher cytolytic (p=0.002) and IFNγ (p=0.01) response. NK-cell response was significantly higher in non-viremic, compared to viremic R+ LTR (p=0.02). In D+/R- LTRs significantly higher NK-cell response was elicited in viremic compared to non-viremic primary infection (p=0.01).

Conclusions: The present data reveal a complex pattern of HCMV–specific AB and NK-cell response in different patient groups post-transplantation.
[O017] IMMUNOLOGIC MONITORING OF CYTOMEGALOVIRUS (CMV)-SPECIFIC T-CELLS RESPONSE IN LUNG TRANSPLANT RECIPIENTS

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Aim: Cytomegalovirus (CMV) remains one of the primary opportunistic pathogens affecting the outcome of lung transplant recipients (LTRs). We evaluated the CMV-specific enzyme-mediated immunity (CMV-CMI) in LTRs comparing the CMV-specific enzyme-linked immunospot* and the QuantiFERON-CMV assay for predicting the onset of CMV infection after stopping antiviral prophylaxis post-transplant.

Method: In this prospective single-center study were recruited 56 consecutive LTRs between February 2017 and August 2018 performed at the Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico. Antiviral prophylaxis was based on donor (D) and recipient (R) serology. The virological and immunological monitoring was established at time 0, 1 and each three months after transplant.

Results: LTRs analysed had a median-age of 35 [25,3-51] years, 39,6% were male. 62,3% LTRs came from cystic fibrosis. Regarding serology status at transplant we observed a excellent concordance with ELISpot (kappa of 0.92) and a moderate concordance with QuantiFERON (kappa 0.59). ELISpot and QuantiFERON presented a good concordance (kappa 0.67). In 24,5% LTRs, QuantiFERON was continnually negative and ELISpot positive.
4 LTRs D+/R- developed HCMV infection during the follow up: 2/4 presented low levels of CMV-CMI whose one deceased with active PTLD. 2/4 presented high levels of CMV-CMI whose one presented Kaposi sarcoma associated to HHV-8 with a good response to chemotherapy and the other one hasn’t presented other infections.

Conclusions: The immunospot* resulted most sensible that QuantiFERON to detect the CMV-CMI. Immunologic monitoring represents a useful tool for identifying LTRs at risk of CMV infection and to adjust the period of prophylaxis.

*ELISpot
Background and Aim: High-resolution DNA (CpG) methylation profiling of cellular genome can quantitatively capture epigenetic states across millions of CpGs and provide insight into gene regulatory circuitries. We aimed to characterize influenza virus specific epigenome response genome-wide in nasal epithelial cells obtained from infected children and controls.

Methods: Whole-genome bisulphite sequencing was applied at 30-40x coverage in pooled nasal epithelial cells from 30 uninfected or influenza infected (Flu A or B) children. Active promoters (UnMethylated Regions) and enhancers (Low Methylated Regions) in each pool were identified using a hidden markov model (HMM) implemented by computational tool*.

Result: On average 80,000 regulatory regions were observed per cell pool with striking influenza-specific activation (hypomethylation) including 15,000 regions unique to FluB whereas only 2000 were uniquely activated in the FluA pool. Further examination of common viral inactivation (hypermethylation) of regulatory elements showed nearly 5000 regions enriched (>10-fold) among genes in immune system activation pathways in patients with Flu A or B vs controls. DNA binding sites for key transcription factors (e.g. STAT1, IRF1, IRF8) involved in mediating either inflammation or interferon response were hypermethylated in infected cells. Asthma associated genetic markers (SNPs from genome-wide association study) were strongly enriched among inactivated regulatory elements.

Conclusion: Genome-wide monitoring of infant viral response provides a catalogue of influenza A and B infection associated host DNA regulatory elements in early childhood. Future assessment of epigenetic variation in these regions in individuals may reveal epigenetic viral triggers for childhood disease.

* MethylSeekR
Aim: “The age-related decline of the immune system, referred to as immunosenescence, increases morbidity and mortality associated with infectious diseases, cancer, cardiovascular disease, and neurodegenerative disease in elderly individuals, as well as weakens vaccination response. The rate of immunosenescence is thought to be affected by certain viruses, such as cytomegalovirus. The collection of all viruses residing in an individual is called the virome of that individual. Knowledge of age-associated changes in the virome and in the immune system could provide opportunities to maintain and enhance the immune system defenses of the elderly.”

Method: “The blood virome compositions of the young and the elderly were compared using RNA sequencing (RNA-seq). Blood samples and measurements of health-related clinical variables were collected from 7 nonagenarians (age 90) and 7 young controls (aged 26 to 32, median age 28), all women. RNA-seq of the samples was performed and the resulting reads were assembled into contiguous sequences (contigs), which were then aligned against a large collection of virus genomes.”

Results: “The matches to viral genomes from each blood sample were compared between age groups and to various clinical variables. Observed differences between age groups in viral matches were less distinct than expected and not statistically significant, except for certain subsets of viruses.”

Conclusions: “These results suggest that the effects of immunosenescence may be specific to the viromes of each individual, and that overarching age-related differences are limited.”
Aim: Metagenomic sequencing can capture the full spectrum of viral pathogens in a clinical specimen and has the potential to become a rapid, all-in-one solution for virus diagnostics. To date, clinical application is still in an early phase and limitations remain. Here, we evaluated the impact of viral metagenomics for cases analyzed over two years in a tertiary diagnostics unit.

Method: Virome analysis was performed upon request by the treating clinician in 71 cases, where the etiology of infection remained unknown or the initial differential diagnosis was very broad. Clinical specimens were analyzed by high-throughput metagenomic sequencing in separate reactions for DNA and RNA viruses. Results obtained by metagenomic analyses were compared to the results and the workload of conventional routine testing.

Results: Over two years, 113 specimens from 71 patients were tested by metagenomic sequencing. The main sample types were blood (30%), cerebrospinal fluid (30%) and throat swabs (12%). In the majority of the investigated cases viral encephalitis/meningitis or respiratory infection was suspected. In parallel, conventional virus diagnostic tests were performed (mean 20 individual tests/patient). Metagenomic sequencing detected viruses in 28 cases (39%). While often confirmatory, in multiple cases the identified viruses were not included in the selected diagnostic tests (e.g. Toscana virus, tick-borne encephalitis virus, Pegivirus C or HIV-1 in cerebrospinal fluid).

Conclusions: Two years of metagenomic sequencing in a tertiary diagnostics unit demonstrated several advantages of an untargeted approach for virus diagnostics, highlighting the potential as first-line diagnostic tool.

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CHARACTERIZATION OF HUMAN DNA VIRUSES PERSISTING IN FEMORAL BONE

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Aim: “Our understanding on viral evolution is mostly derived on prediction models rooted on the examination of current viral diversity alone. Thus, the study of viruses from historical sources, even over short timescales, is essential to provide a higher calibration and resolution for the assessment of viral emergence, fitness and adaptation. As most ancient human remains and museum specimen are skeletonized, it is of utmost importance to evaluate viral DNA occurrence in bones. We aim at characterizing the DNA of human viruses persisting in bones.”

Method: “Cortical femoral bone samples were collected from 32 recently deceased individuals. The DNA was extracted using an accredited method of known efficiency for downstream DNA analysis from bone. The viral genomes were investigated using i) in-house quantitative PCRs (for human parvovirus B19 (B19V), Epstein-Barr virus (EBV) and torque teno-virus (TTV)), and ii) in-solution-capture enrichment (multiplex detection of persistent/latent human DNA viruses) with virus-specific biotinylated RNA baits followed by next-generation sequencing (NGS) in the Illumina platform.”

Results: “Viral DNAs were detected in human femoral bone with prevalences of 73, 60 and 20 % for TTV, B19V and EBV, respectively. Sequences of the families Parvoviridae, Anelloviridae, Polyomaviridae and Herpesviridae were identified and characterized.”

Conclusions: “This work expands our knowledge of viral bioportfolio to human bone and yields a novel NGS-based approach for the detection of DNA viruses in contemporary and archival human tissues. Thus, bone may withhold an invaluable window to the past, providing evidence on viral origin and evolution.”
[O022] THE RESPIRATORY VIROME AND EXACERBATIONS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Aim: Exacerbations are major contributors to morbidity and mortality in patients with chronic obstructive pulmonary disease (COPD), and respiratory infections are an important trigger. Our aim was to study the respiratory virome in nasopharyngeal samples during COPD exacerbations using mNGS.

Method: 88 nasopharyngeal swabs from 63 patients from the Bergen COPD Exacerbation Study (2006-2010) were analysed by mNGS and in-house RT-PCR for respiratory viruses. Both DNA and RNA were sequenced simultaneously using an Illumina library preparation protocol with in-house adaptations.

Results: By mNGS, 23/88 samples tested positive. Sensitivity and specificity when compared to PCR varied between 92%-96% and 96%-100% respectively using different cut-off values for the number of sequences reads defining a positive result. A ROC curve demonstrated optimal sensitivity and specificity using a cut-off of approx. 10 reads. Additional pathogens detected by mNGS were herpes simplex virus type 1 and coronavirus OC43. Patients with viral pathogens had lower percentages of bacteriophages compared to patients without viral pathogens 17% and 54% respectively (p<0.001). Furthermore, Shannon diversity scores were compared.

Conclusions: The mNGS protocol used was highly sensitive and specific for semi-quantitative detection of respiratory viruses. Excellent negative predictive value implicates the power of mNGS to exclude any infectious cause in one test, with consequences for clinical decision making. Reduced abundance of bacteriophages in COPD patients with viral pathogens implicates skewing of the virome during infection, with possible consequences for the bacterial populations, during infection.

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ATYPICAL FATAL ENCEPHALITIS MEASLES DETECTED AND CHARACTERIZED BY STANDARDIZED AND VALIDATED SHOTGUN METAGENOMIC APPROACH

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Aim: Shotgun metagenomics (SMg) have demonstrated high potential to detect all pathogens in undiagnosed syndromic infectious disease. Thus, we have developed an accredited SMg technology to explore atypical and severe clinical cases. We report a case of a 28-year-old HIV-positive woman, twice-admitted to the emergency department for neurological syndrome, treated as epileptic disease. The clinical worsening of the patient associated with uncontrolled HIV replication (3.65 log cp/mL) and low CD4 T-cell count (26/mm3) lead to intensive care unit hospitalization. Despite antiretroviral therapy, the patient falls into a coma and subsequently died with encephalitis symptoms. MRIs concluded to probable severe status epilepticus and all conventional microbiology testing assays remained negative on clinical samples including sequential cerebrospinal fluids and cerebral biopsy which were tested by SMg.

Methods: SMg approach included pan-pathogen nucleic acid extraction, RNA/DNA library preparation, sequencing**, and analysis with the software* which performed microorganism identification, genome reconstruction, and variant calling.

Results: Measles virus was detected in the cerebral biopsy with nearly-complete genome assembly (99.5% with depth coverage median >50,000). Phylogenetic analyses reveal MeV B3-genotype and no known mutation accounting for neurovirulence were identified. Results were also confirmed by specific MeV qRT-PCR performed by the National Reference Lab for Measles. The final diagnostic was a Measles inclusion-body encephalitis (MIBE).
Conclusion: The patient harbored a measles infection with no skin rash which leads to misdiagnosis of a MIBE. This case highlighted the major advantage to have a reliable SMg tool to diagnose atypical disease without evident etiology.

*MetaMIC® (ISO-EN-NF-15189)
**NextSeq®500(Illumina
Aim: Enteroviruses (EV) are small RNA viruses associated with a variety of symptoms, from mild illness to severe neurological infections. At the Institute for Infectious Diseases, University of Bern, a bio bank of patient samples screened for EV presence by the diagnostic department was collected since 1998. This enabled an epidemiological analysis of EV cases of the last 20 years and surveillance of circulating genotypes from recent years.

Method: For the epidemiological analysis, number of EV cases was related to demographic information, specimen type, across time and, when available, associated symptoms and genotypes. Additionally, genotyping of >50 cases from 2017 and 2018 was performed from patient samples by sequencing of the surface protein VP1 encoding gene using nanopore sequencing and validated using Sanger sequencing.

Results: From 1998 to June 2018, more than 2000 samples were found to be positive for enterovirus. They were most commonly detected in cerebrospinal fluid (52%) and stool (30%) specimens. Most enterovirus-positive cases were associated with neurological symptoms (55%; mainly meningitis), followed by fever (40%) and gastrointestinal symptoms (14%). Sequencing revealed a diverse range of genotypes (with Echovirus 30 most common), of which the majority belong to species B and most remaining to species A. The comparison of Nanopore sequencing of multiplexed amplicons to Sanger sequencing confirmed >99% sequencing accuracy.

Conclusions: This study gives insights on the extent and types of enterovirus infections in one of the largest Swiss hospitals over the last 20 years and an overview of recently circulating genotypes in Bern, Switzerland.
Aim: Here we describe a cluster of rotaviruses G2P[4] infections among adults in a university hospital in Germany and report the outbreak investigation.

Method: Rotaviruses are known to infect mainly young children. In adults, symptomatic rotavirus infections are less common, and outbreaks have been described only sporadically. A rotavirus outbreak among adult patients was detected between December 2016 and April 2017 in the university hospital of Freiburg, Germany. Rotavirus RNA was detected by using real-time RT-PCR and positive samples were typed in the VP4 (G-type) and VP7 (P-type) genes.

Results: A total of 33 outbreak-associated rotavirus cases were identified, of these, two asymptomatic patients were detected through point-prevalence screening on one affected ward. Median age of patients was 66 years, 20 were males and 14 were immunosuppressed. The detection rates peaked in calendar weeks 2-4. The majority of cases were identified in wards A (n=9), B (n=6), and C (n=5). The remaining 13 cases were scattered across the university hospital. Genotyping was successful in 91% of samples and all cases belonged to rotavirus G2P[4] genotype. Of note, one rotavirus G3P[8] infection was detected in calendar week 14 suggesting independent introduction from the community. Two additional cases were detected in calendar week 16 resembled the G2P[4] genotype which were, however, slightly different from the hospital-associated strain on the nucleotide level.

Conclusions: We detected a nosocomial outbreak of rotavirus genotype G2P[4] among adults. Despite intensive work-up the source of the outbreak was not identified. Increased awareness seems important to detect rotavirus outbreaks.
[0026] METAGENOMIC IDENTIFICATION AND WINTER SEASONALITY OF PICOBIRNAVIRUS IN STOOL OF HOSPITALIZED PATIENTS WITH ACUTE GASTROENTERITIS

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Aim: To use metagenomic next-generation sequencing (mNGS) to identify the causative agent in patients hospitalized for acute gastroenteritis with unknown etiology.

Method: A total of 300 stool samples collected from in-patients with gastroenteritis from April to June 2018 were investigated (discovery cohort). These samples had tested negative for common diarrheagenic bacterial and viral pathogens. Nextera XT libraries targeting virome were prepared and sequenced on NextSeq 500. Taxonomic classification of sequencing reads was performed using Taxonomer. Seasonality and prevalence of any newly identified viruses were studied in an independent cohort of 246 stool samples collected from December 2018 to February 2019 (validation cohort).

Results: In the discovery cohort, 10 (3.3%) samples were positive by mNGS for picobirnavirus, a RNA virus currently not included in any in-house diagnostic workflows and commercial gastrointestinal virus testing panels. On-target reads ranged from 0.06%-8.5%. In the validation cohort, 15 (6.1%) samples were positive for picobirnavirus by a virus-specific RT-PCR assay. The virus was present in both children and adults but had a higher prevalence towards middle-aged adults: 0-4 years (0%; 0/73), 5-40 years (7%; 2/29), 41-64 years (13%; 8/63), 65-84 years (7%; 4/54), and ≥85 years (4%; 1/27). Picobirnavirus exhibited a winter seasonality typical to other gastroenteritis viruses: 3% (3/90) in December 13% (10/80) in January, and 2% (2/76) in February.

Conclusions: mNGS identified picobirnavirus in gastroenteritis patients with unknown etiology. The winter seasonality implies picobirnavirus is not opportunistic and may be pathogenic and cause gastroenteritis.

Acknowledgements: This study was supported in part by HMRF CU-17-B8.
Aim: Viral triggers of coeliac disease (CD) have not been investigated using molecular methods applied on longitudinal cohorts, with an exception of our earlier report on enterovirus. Here we aimed to test whether another frequent human enteric virus, Human parechovirus (HPeV), is associated with the development of coeliac disease (CD).

Method: During 2014-16, 237 children with HLA-DQ2/DQ8 from the longitudinal birth cohort study MIDIA consented to CD screening, with 25 children diagnosed with CD. For each case, two randomly selected controls were matched. We retrospectively analysed stored serial blood samples for CD antibodies (CDa) and identified the CDa seroconversion interval. Then we tested HPeV with quantitative real-time RT-PCR in 2005 stool samples collected monthly from 3-36 months of age. The data were analysed using mixed effects logistic regression models with faecal virus positivity before seroconversion of CDa as the dependent variable and CD status as an independent variable, adjusted for potential confounding factors.

Results: HPeV was detected in 222/2005 stool samples (11.1%) and was more frequent before subsequent CDa seroconversion (adjusted odds ratio [aOR] 1.69, 95% CI 1.15-2.46). This association was stronger if diarrhea was present, but not other symptoms (aHR 3.72, 95%CI 1.03-13.42). Estimates were essentially unchanged when restricted to infections after gluten exposure. Concurrent positivity for HPeV and enterovirus was strongly associated with subsequent CD (aHR 4.58, 95%CI 1.25-16.69).

Conclusions: Our findings suggest that parechovirus and enterovirus may either significantly contribute to CD development or be an important marker of the earliest stages of the gut injury.
Aim: In the past decade, the number of people able to travel and the distance they travelled raised significantly. This is associated with an increased infection risk. Some infections can be prevented by vaccination, as recommended by international guidelines. However, not all travellers are aware of these recommendations. The Dutch travel Vaccination Study (DiVeST) aimed to study adherence to travel health guidelines in families.

Method: Travellers who visited an Eastern- or non-European country in the preceding year were recruited via Dutch secondary schools between 2016-2018. Vaccination status was assessed by questionnaires and vaccination records. Subsequently, we choose hepatitis A virus (HAV) vaccination as representative travel vaccination and studied antibody concentrations with an enzyme linked immunosorbenent- and chemiluminescent immune assay in dried blood spot eluates.

Results: Of the 246 travellers that participated in this study, 63% travelled to destinations were HAV vaccination was recommended. Of these 155 travellers, 56 (36%) reported a visit to a travel clinic prior to their trip, and 64 individuals (41%) showed proof of a valid HAV vaccination in their vaccination records. In 98 out of 145 (68%) travellers anti-HAV antibodies were detected.

Conclusions: Overall, a majority of travellers do not visit a travel clinic prior to their travel and do not have their vaccination records well documented. HAV seroprevalence among people who travelled to destinations with HAV vaccination recommendation was low. To increase adherence to travel health guidelines specific attention should be given to subgroups, identified by DiVeST, whom are unaware or unmotivated to be vaccinated.
<table>
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<th>Age groups</th>
<th>Seronegative travellers (%) n = 47</th>
<th>Seropositive travellers (%) n = 98</th>
<th>Total travellers (%) n = 145</th>
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Aim: Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Our goal was to characterize the prevalence of High-Risk HPV genotypes in university students at the Northeast region of Portugal using self-collection samples.

Method: A total of 81 university students were given self-sampling systems for cervical-vaginal samples collection. High-Risk HPV genotyping was performed using platform*.

Results: The median age of women was 21.0 years (range 18-30) and mean age of first sexual intercourse was 17±1.9 years. High-Risk HPVs were detected in 31 women (38.3%), with single and multiple infections to be responsible for 16.0% and 22.3%, respectively. Overall, HPV-68 (9.9%) was the most frequent genotype, followed by HPV-31, -51, -58, -59 and -66 each with 7.4%. We also observed that three women had HPV-16 and one HPV-18. We observed that women reporting more than two lifetime sexual partners, first sexual intercourse under 17 years old, non-Portuguese nationality (mainly African origin) and non-vaccinated status were associated with higher prevalence of High-Risk HPVs (p=0.001, p= 0.014, p=0.005 and p=0.016, respectively).

Conclusions: Our study revealed that 1) self-collecting samples are useful for the detection of High-Risk HPVs; 2) the prevalence of High-Risk HPV infection in university students in the Northeast region of Portugal is high, particularly in those with over 21 years of age of non-Portuguese nationality. These results highlight the importance of continuing to develop prevention strategies and that self-collected samples may be a useful sample in the context of HPV-detection

* the AnyplexTM II HPV HR Detection (Seegene®)
QUASISPECIES DIVERSITY ACCELERATES MORBILLIVIRUS ADAPTATION TO NEW ENVIRONMENTS

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Aim: “The Morbillivirus genus includes human and veterinary negative-sense, single-strand RNA viruses with high clinical relevance and worldwide distribution. Morbilliviruses are subject to genetic constraints, resulting in efficacy of live-attenuated vaccines developed 50 years ago. As RNA viruses, they have considerable adaptation potential, but the contribution of quasispecies and de novo mutations to this potential remains elusive. Towards this, we performed an adaptation experiment with recombinant and natural canine morbilliviruses in ferrets.”

Method: “Both strains underwent three consecutive in vivo passages, followed by assessment of the clinical course of disease and viral load kinetics. To analyze genetic changes, RNA sequencing was performed on input and passaged viruses.”

Results: “Passaging resulted in an increase in clinical signs and mortality for both viruses, even though reduced dissemination was initially observed for the recombinant virus. Coding mutations found in the passaged natural virus reproduced those identified in a previous adaptation with the same virus. RNA sequencing revealed that some mutations of the natural virus were quasispecies-derived, while all changes in the recombinant virus originated from de novo mutations. Even though a reduced diversity was seen in the recombinant input virus, the total diversities of both strains after passaging were more equivalent but shared only a small number of variant sites and one coding mutation.

Conclusions: “Adaptation of Morbilliviruses results in reproducible genetic changes in the context of similar selection pressures. A higher initial diversity benefits adaptation, but these viruses are also capable of acquiring de novo mutations to adapt and increase pathogenesis.”
MUMPS VIRAL LOADS - ASSOCIATION OF CT VALUES WITH AGE, GENDER AND DOSES OF MMR VACCINE.

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¹National Virus Reference Laboratory, University College Dublin, Dublin, Ireland

Aim: Although the diagnosis of mumps is primarily based on clinical symptoms, other viral infections such as parainfluenza can manifest in a similar manner. Therefore, laboratory confirmation of mumps infection is important. Detection of mumps RNA in oral fluid samples is rapid with high sensitivity and specificity. The aims of this study were to determine whether mumps viral load differed in outbreak and non-outbreak periods, and was associated with age, gender or doses of MMR vaccine received.

Method: All oral fluid samples requested for mumps RNA testing from Jan 2018 to March 2019 (n=1296) were included in the study. A mumps outbreak began in Ireland in August 2018 and is currently ongoing. The mumps N gene was detected by real time PCR and reported as Ct values.

Results: Mumps RNA was detected in 490 samples (37.8%). The proportion of samples received monthly with detectable mumps RNA increased from 10.26% up to 70.3% during the recent outbreak. Acute mumps cases occurred predominantly in the 16-25 year old age cohort (67.5%) and in males (55.9%). Ct values were significantly higher in males (p<0.001). During the outbreak, a significantly higher proportion of samples had Ct <30 (p<0.05). The majority of samples were from individuals who had received 2 doses of MMR vaccine.

Conclusions: Our findings show that mumps virus is maintained in circulation in the non-outbreak period. Acute mumps cases occur predominantly in the MMR vaccinated young adult male population.
Background: “Human cytomegalovirus (HCMV) is a risk for anyone whose immune system is undeveloped or impaired. Infection can involve multiple HCMV strains, which can infect various organs differentially. Thus, the strain present in blood, which is conventionally used for diagnosis, might differ from the strain damaging a particular organ. Identifying the strains associated with disease is critical for implementing the most effective treatment. HCMV compartmentalization has been explored previously by focusing on a few viral genes, leaving this area ripe for exploitation in the age of high-throughput DNA sequencing (HTS)”

Aim: “To examine the occurrence of multiple HCMV strain infections (MSIs) and HCMV compartmentalization in the respiratory system of HCMV-infected patients using HTS”

Method: “Paired samples (plasma/blood and bronchoalveolar lavage) were collected from lung (LT; n=10) and hematopoietic stem cell (HSCT; n=7) transplant recipients, and HIV-infected children with HCMV pneumonitis (HCMVp; n=12). Sequencing libraries were generated following a target enrichment protocol and sequenced on a instrument*. MSIs were assessed by screening the sequencing data for genotype-specific motifs from the 12 most variable HCMV genes”

Results: “MSIs were detected in 40% of HSCT and 70% of HCMVp patients. Different degrees of compartmentalization were detected, mainly as different proportions of the HCMV strains in both compartments. Some cases presented unique strains in one compartment”

Conclusions: “The application of HTS in paired clinical samples revealed the common occurrence of MSIs and HCMV compartmentalization in HSCT and HCMVp patients. It is possible that this is associated with the level of immune suppression received”

* NextSeq
[O033] PREVALENCE OF ALL KNOWN HUMAN POLYOMAVIRUSES DETERMINED BY SEROLOGY AND PCR IN 1000 HEALTHY BLOOD DONORS

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Introduction: The number of human polyomaviruses (HPyVs) has increased to fourteen. In immunocompromised patients, HPyVs are known to cause disease, such as nephropathy (BKPyV), progressive multifocal leukoencephalopathy (JCPyV) and skin cancer (MCPyV), while healthy individuals remain asymptotically infected. Whether latent HPyVs circulate in blood (viremia) and are potentially infectious, for example for transfusion-receiving immunosuppressed patients, is largely unknown.

Aim: To determine the (sero)prevalence and load of all known HPyVs in healthy individuals

Methods: Quantitative PCR and multiplex serology to detect HPyV-specific DNA and VP1 IgG antibodies, respectively, was performed on serum from 1016 Dutch blood donors.

Results: Seroprevalence of most HPyVs was high (60-100%), except for HPyV9 (~20%) and HPyV12, NJPyV and LiPyV (<5%). The median number of HPyV coinfections was nine. HPyV DNA in blood was detectable in 5% of the population, with a maximum viral load of 452 copies/ml. The most prevalent polyomavirus was MCPyV, detected in 39 samples (3.8%), followed by JCPyV (0.5%), TSPyV (0.5%) and HPyV9 (0.4%). Amplicon sequencing confirmed the presence of viral DNA in most cases. For JCPyV a strong correlation was observed between detection of viral DNA and height of the seroresponse.

Conclusions: Polyomavirus (co)infection is common in healthy individuals and occasionally accompanied by viral DNAemia, including pathogenic viruses such as JCPyV. Further study is needed to determine the presence and infectivity of HPyV in donor blood products, and the potential risk of HPyV infection for immunocompromised recipients.
LONGITUDINAL MONITORING OF HCMV-SPECIFIC T-CELL RESPONSE IN KIDNEY TRANSPLANT RECIPIENTS DURING THE FIRST YEAR AFTER TRANSPLANT

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Aim: The aim of the study was to evaluate the role of immunological markers in monitoring kidney transplant recipients (KTRs) at risk for HCMV infections.

Method: Eighty-six KTRs were prospectively enrolled and monitored for at least six months post-transplant. Pre-transplant HCMV serostatus was assessed, HCMV DNAemia and HCMV-specific T-cell response was monitored in the post-transplant. Ex-vivo ELISpot assay was performed using peptide pools representative of pp65, IE1 and IE2 proteins, for evaluation of HCMV-specific T-cell response. HCMV DNAemia was measured in blood samples according to diagnostic protocols.

Results: Among 86 enrolled patients, 7 (8,1%) patients were D-/R- and they did not showed episodes of HCMV infection. Fourteen patients of 86 (16,3%) were D+/R- and 11 of them (78,6%) were treated for clinically relevant HCMV infection. HCMV-specific T-cell response was undetectable during the first three months post-transplant. A significantly increase in pp65- and IE2-specific T-cell responses was observed after 180 days post-transplant, followed by IE1-specific T-cell response. Sixty-five patients of 86 (75,6%) were R+ at transplant and 22 of them were treated for clinically relevant HCMV reactivations (33,8%). Interestingly, 7/65 patients (10,8%) did not showed any episode of HCMV reactivation during the follow-up period. In this latter group of patients IE1-specific T-cell response was significantly higher at pre-transplant than respect to other R+ patients (p=0.0177). Deficient IE1-T cell response was observed in treated patients.

Conclusions: IE1-specific response represent a useful immunological marker of risk for clinically relevant HCMV infections. Further analysis is required on larger population for definition of clinical cut-off.
[O035] EXPLORING TTV LOAD KINETICS TO PREDICT ALLOGRAFT REJECTION AND VIRUS INFECTION IN KIDNEY TRANSPLANTATION PATIENTS

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Aim: “Torque teno virus (TTV) is a ubiquitous, non-pathogenic virus controlled by the immune system. TTV DNA load has been proposed as a universal biomarker of effective immunity in solid organ transplantation (SOTx) recipients. Here we explored TTV load kinetics as a predictor of infection and allograft rejection in kidney transplantation (KTx) patients.”

Method: “TTV loads were determined by qPCR in an existing cohort of consecutive blood samples from 389 KTx recipients routinely collected within one year after KTx. Patient, transplantation and treatment characteristics were compared with TTV load kinetics. First episode of BK polyomavirus (BKPyV) viremia and of allograft rejection treatment were compared with TTV load kinetics using a survival model with mixed effects.”

Results: “During follow-up, TTV detection rapidly rose to 100% and median viral load increased from approximately $10^4$ to $10^7$ copies/ml, peaking 12 weeks after KTx. BKPyV viremia developed in 27% and allograft rejection in 23% of recipients. Underlying glomerular disease and tacrolimus use were more common among patients with high TTV loads. Overall, with every log₁₀ TTV load increase, the risk of BKPyV viremia increased (HR 1.08, CI 1.09-1.13), while the risk of rejection decreased (HR 0.85, CI 0.82-0.88). Preliminary analysis showed that patients with the highest TTV loads during the first 6 weeks did not develop rejection during the remaining follow-up time.”

Conclusions: “Changes in TTV load after KTx predict BKPyV viremia and allograft rejection in opposite fashion. Usefulness of individual TTV load monitoring, especially in the first weeks after SOTx, deserves more study.”
Aim: CMV is the leading infectious cause of neurological impairments in newborns. However, universal prenatal screening for CMV infection is not recommended by European or American obstetric societies. We hypothesize cell-free DNA (cfDNA) from a clinically-implemented prenatal aneuploidy screen can detect CMV DNAemia during pregnancy.

Method: cfDNA sequences were obtained from 2,210 patient samples using a validated, laboratory-developed sequencing and data pipeline for fetal aneuploidy detection. The paired-end cfDNA sequences were aligned to the human herpesvirus 5 Merlin strain (NC_006273.2) using bowtie2. Reads were confirmed via BLASTn alignment to the NT viral database. Samples were stratified based on CMV reads per million (RPM). An RPM >0.4 was considered high positive and 0< RPM <0.4 as intermediate. High positive and a subset of intermediate positive with run-matched negative samples were tested for CMV by qPCR using DNA extracted during the cell-free procedure.

Results: CMV reads were identified in 10 samples as high positive and 150 as intermediate positive. Quantitative PCR detected CMV in all high positive samples and 2 of 32 intermediate samples tested (range: below limit of quantitation to 434 copies/ml). CMV RPM value demonstrated a linear correlation to viral load by qPCR. The median CMV cfDNA insert size was significantly shorter than human cfDNA insert size (140bp v. 170bp, p<0.001). CMV reads were not concentrated to any specific region of the CMV genome.

Conclusions: Cell-free DNA compares favorably to qPCR for the detection of CMV and may provide a novel screening method for prenatal infectious diseases.
Aim: The aim of the study was to explore the prevalence and significance of parvovirus B19 (B19V) DNAemia in pregnancy.

Method: The study population consists of women included in the Norwegian Mother and Child Cohort Study, a prospective population-based pregnancy cohort. Blood samples were obtained from mothers during week 17-18 in pregnancy (M1) at birth (M2) and in umbilical cord blood (C). A total of 1350 controls and 138 cases of perinatal death were included. We performed B19V-PCR and serology in every available M1-sample from cases, and a subset of 149 randomly selected controls.

Results: The B19V-PCR results of the M1-samples in cases and the subset of controls showed high and equal prevalence of B19V-PCR positive M1-samples, 24.0% and 28.2% respectively. In both cases and controls, nearly half of the PCR positive samples were seronegative for B19V-IgG and -IgM antibodies in the M1-sample. Among controls in M2 only three of thirteen PCR positive samples were seronegative, of which two had a high level of viremia. The prevalence of positive B19V-DNA in available C-samples was 9.1% in cases and 11.9% in controls.

Conclusions: Similar high prevalence of B19V-PCR positive samples were recorded in both cases and controls. The clinical significance of B19V-detection during pregnancy is uncertain and warrants caution based only on detecting B19V-DNAemia.

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Aim: In 2018, five European countries reported an increase in echovirus 30 (E30) infections. We explored the genetic characteristics of the virus strains detected prior and during the outbreak.

Method: The EU/EEA countries were invited by ECDC to submit E30 VP1 sequences (>200 bp) from years 2016-2018. Sequences were analysed using Simple Sequence Editor and phylogenetic trees built using Molecular Evolutionary Genetics Analysis 7.0.

Results: 1688 E30 VP1 sequences were reported by 22 European countries. 1182 sequences were of acceptable quality for phylogenetic analysis, most of these obtained from cerebrospinal fluid samples (809/1182; 68%). Sequences were divided into eight phylogenetic groups (G1-G8) based on 5% nucleotide divergence. Most sequences belonged to G1 (n=687) followed by G6 (n=263), G2 (n=166), G4 (n=56), G8 (n=4), G3 (n=3), G5 (n=2) and G7 (n=1). G2 viruses dominated in 2016 (128/250; 51%), whereas most sequences obtained in 2017 and 2018 belonged to G1 (279/456; 61% and 330/476; 69%, respectively). Country variation was observed: G1 was identified as the most prominent genetic lineage in the United Kingdom, Ireland, Norway and Germany already in 2016 and 2017. In 2018, G1 viruses continued to circulate elsewhere in Europe but were replaced by G6 viruses in the United Kingdom (92/142; 65%).

Conclusions: This study describes genetic diversity of E30 outbreak strains and the temporal and geographical dynamics of individual phylogenetic groups. These are important to monitor to understand causes of the outbreak as well as outcomes of it. Further analysis will illuminate the relationship of recombination events in genotype replacements.
[0039] MOLECULAR ANALYSIS OF AN ONGOING MEASLES OUTBREAK IN ISRAEL RELATED TO MULTIPLE SIMULTANEOUS IMPORTATION.

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Aim: Israel continues to struggle with an ongoing nationwide outbreak of measles. On March 15, 2018, an index case of an Israeli man returning from Thailand was diagnosed. Two weeks later secondary measles developed endemically, all had visited or worked in healthcare facilities that the index case had visited. Through April 2019, more than 3,900 measles cases were diagnosed. More than half of the cases were laboratory-confirmed, and the rest were epidemiologically linked to the confirmed cases. Of the laboratory-confirmed cases, more than 80% were diagnosed by Real-time PCR. Each imported case can potentially initiate a new chain of transmission, resulting in several simultaneous outbreaks of unrelated strains which are difficult to differentiate with standard epidemiologic investigation. In order to validate this assumption, expanded molecular analysis of the confirmed cases must be performed and compared to the results of the epidemiologic investigation.

Method: Altogether 250 cases were sequenced with both the N-450 region and the hypervariable region M/F-NCR of Measles virus.

Results: Sequencing of the N-450 region revealed that all cases were of genotype D8. Most cases were similar or even identical to each other, suggesting that this region can reveal only limited information on the chain of transmission. Thus, further sequencing of the virus hypervariable region M/F-NCR was performed.

Conclusions: Sequencing of the hypervariable region of the viral genome can increase the resolution of the molecular analysis and will be critical in elaborating outbreak chains of transmissions.
[0040] ENTEROVIRUS D68 SEROSURVEY REVEALS EVIDENCE FOR ENDEMIC CIRCULATION IN THE NETHERLANDS

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Aim: Enterovirus D68 (EV-D68) was infrequently detected before 2010, when it started causing major outbreaks of severe respiratory illness worldwide. Especially young children and individuals with underlying conditions are at risk of developing severe lower respiratory tract disease. EV-D68 has the potential to spread to the central nervous system, and acute flaccid myelitis has been strongly associated with EV-D68 infection. In 2018, 2 cases of EVD68-related AFM were reported in the Netherlands. Here, EV-D68 circulation in the Netherlands was evaluated before and after 2010 by conducting a serosurvey.

Method: We screened 140 sera from Dutch children and adults from two independent sets of samples collected during 2006/07 and 2015/16, timepoints prior and post to 2010 when the first EV-D68 upsurge in the Netherlands was observed. Neutralizing antibody (nAb) titer in the sera was tested by virus neutralization assays against the prototype Fermon EV-D68 strain, isolated in 1962, and against a recent EV-D68 strain (genotype B3) isolated in 2016.

Results: We found remarkably high overall seropositivity (94.3-98.3%) for nAbs against both EV-D68 strains, regardless of the time of serum collection. Age-stratified analyses indicated that the overall EV-D68 nAb seroprevalence was approaching 90% already in children 1-10 years old. Geometric mean nAb titres increased in an age-dependent manner.

Conclusions: Our data show that EV-D68 has been circulating in the Netherlands for decades and that the EV surveillance underestimates the prevalence of this clinically relevant pathogen. Serosurveillance can be a valuable tool for monitoring virus circulation in the population.
Aim: Epstein-Barr virus (EBV) has been associated with almost 10% of gastric carcinomas, although its role in gastric carcinogenesis remains unclear, especially because the detection of EBV in premalignant lesions of gastric cancer is extremely controversial. This study intends to determine the prevalence of EBV in gastric dysplasia and superficial neoplasia to clarify whether EBV infection is an early or late event in gastric cancer development.

Method: We developed a retrospective study included 199 consecutive patients (242 gastric lesions) referred for endoscopic resection between March 2003 and December of 2015 at Portuguese Oncology Institute of Porto. EBV infection was detected in histological sections obtained from formalin-fixed paraffin-embedded (FFPE) tissue blocks using EBER-ISH.

Results: Histological classification showed a total of 37 (15.3%) low-grade dysplasias, 100 (41.3%) high-grade dysplasias, 78 (32.2%) intramucosal carcinomas and 27 (11.2%) submucosal carcinomas. EBV was not detected in epithelial cells of any case with dysplasia or superficial carcinomas, nevertheless we found a small number of EBV-infected lymphocytes in 2.1% of all lesions (4 cases of low-grade dysplasia and 1 case of high-grade dysplasia).

Conclusions: These results showed that EBV is not present in gastric dysplasia neither in superficial carcinomas suggesting that EBV infection is a late event in gastric carcinogenesis.
Aim: Human endogenous retroviruses (HERV) are remnants of exogenous retroviral infections, representing 8% of the human genome. Their regulation is based on the DNA methylation of promoters, the long terminal repeats (LTRs). Transcripts from HERV have been associated with cancers, but reports concerning HERV expression in colorectal cancer remain sporadic.

Method: Sixty-three patients with advanced stages of colorectal cancer were enrolled in this study. HERV-H, -K, -R and -P, LTRs, and Alu, and LINE-1 methylation levels, and the expressions of HERV env gene were investigated by RT qPCR in the tumor, normal adjacent tissues, and, when possible, blood and plasmatic extracellular vesicles (EVs). The expression of the HERV-K Pol protein was also evaluated by Western Blot.

Results: Alu, LINE-1, HERV-H and -K LTRs were demethylated in the tumor compared to the normal adjacent tissues (p<0.05), while no differences were observed in HERV env gene expression levels among the clinical specimens. The env gene was expressed in the EVs (p=0.01) of 54% (-H), 38% (-K), 31% (-R) patients. HERV K Pol protein was more expressed (p=0.0013) in the adjacent normal tissues compared to the tumor tissues.

Conclusions: The changes in DNA methylation of retroelements is specific in colorectal cancer but does not correlate with viral overexpression. The Pol protein expression in the normal cells may induce the retrotranscription and the subsequent transfer of HERV sequences into other cells, possibly through EVs. HERV genome insertion might cause cells transformation.
ANNEXIN II AS DENGUE VIRUS 2 BINDING PROTEIN MEDIATING VIRUS INTERACTION WITH FILOPODIA AND INTERNALIZATION INTO HOST CELLS

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Aim: “Recent evidence demonstrated that dengue virus 2 requires an active filopodia formation for successful infection. The present study was aimed at elucidating dengue virus binding protein on filopodia which is responsible for enhanced infection.”

Method: ”Confocal and Scanning electron microscope were utilised to visualised filopodia formation induced by dengue virus 2. Virus overlay protein binding assay (VOPBA) and LC-MS/MS were used to identify dengue virus 2 binding protein. Confocal microscopy, western blotting and flow cytometry were employed to demonstrate annexin II extracellular translocation in Vero cells. While antibody-mediated infection inhibition and siRNA-mediated knockdown of annexin II expression were used to demonstrate the role of annexin II in dengue virus 2 infection. Colocalization analysis, co-immunoprecipitation and molecular dynamic simulation were employed to demonstrate dengue virus 2 E-glycoprotein interaction with annexin II.”

Results: “The result identified annexin II as dengue virus interacting protein, expressed on the surface of filopodia induced cells. Upon filopodia formation annexin II were observed to translocate to the external leaflet of plasma membrane of the cell. Antibody-mediated infection inhibition assay and siRNA-mediated knockdown of annexin II expression significantly reduces dengue virus 2 infection and production level. Furthermore, colocalization analysis showed extracellular and intracellular colocalization between annexin II and dengue virus 2 E-glycoprotein. And the interaction between annexin II and dengue virus 2 E-glycoprotein was confirmed by co-immunoprecipitation and molecular dynamic simulation.”

Conclusions: “Collectively, we demonstrated for the first time that annexin II mediates dengue virus 2 binding and internalization into Vero cell via filopodia.”
EPIDEMIOLOGY OF HTLV-INFECTED BLOOD DONORS IDENTIFIED IN THE UK BETWEEN 2002 AND 2017 – STILL A LARGELY UNDIAGNOSED DISEASE ENTITY?

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Aim: Although most HTLV-infected individuals remain asymptomatic, HTLV can lead to adult T-cell leukaemia/lymphoma (ATLL) and HTLV-1 associated myelopathy (HAM). Here we describe the epidemiology of HTLV-infected blood donors identified in the UK since the screening was introduced in 2002.

Method: Epidemiological information for all HTLV-infected blood donors was reviewed. Individuals with a reactive HTLV-antibody test were considered as confirmed if Western blot or proviral PCR was positive.

Results: A total of 254 HTLV-positive blood donors were identified in the UK between 2002 and 2017, with a prevalence of 0.7 per 100,000 donations. Most of them were women (182/254,72%), UK-born (125/254;49%) and associated with HTLV-1 (228/254;90%). Mean age was 43 years. Almost all positive donations were made by previously HTLV-untested donors (240/254), but seroconversion within a year from previous donation was confirmed for 5 of the 14 previously tested donors. Most HTLV infections were associated with endemic countries (including the Caribbean region, West Africa, Iran, India and Japan), and were likely acquired through breast feeding or heterosexual partner. Interestingly, three blood donors were thought to have been infected through self-flagellation. A total of 195 HTLV-positive asymptomatic individuals were consented to the HTLV National Register, and during the 841-person year follow-up, although none were diagnosed with ATLL or HAM some reported symptoms that may be associated.

Discussion: This study highlights the potentially large number of undiagnosed HTLV infections in the UK, mostly within UK-born individuals, and evidence of ongoing transmission. The issues around different HTLV screening tests will also be discussed.
Aim: Continuous surveillance of influenza in swine populations plays an important role in reducing the probability of interspecific adaptation and spread of influenza viruses, minimizing the role of these animals as the source of the next pandemic agent. Molecular genetic examination, along with the studies on the biological (antigenic) properties of influenza virus, are an important component of viral variability monitoring, since they allow for tracking the appearance of mutations responsible for changing the functional properties of viral proteins, including those that increase the pathogenicity of the causative agent.

Method: 712 nasopharyngeal swabs were collected in 2018 from swine at livestock farms and private farmsteads located in the Aktobe, Kostanay, North Kazakhstan, East Kazakhstan, Karaganda, and Almaty oblasts of Kazakhstan.

Results: The primary screening of biological samples in RT-PCR using the AmpliSens reagents (Moscow, Russia) demonstrated that the genetic material of influenza A virus was detected in 215 nasopharyngeal swabs (30.20% of the total number of examined samples). Subtyping showed the presence of influenza A/H1N1 virus RNA in 67 samples (31.16%), and influenza A/H3N2 virus RNA in 52 samples (24.19%).

Conclusions: The results of examining samples collected in 2018 from various regions of the Republic of Kazakhstan in RT-PCR therefore indicate co-circulation of influenza A/H1N1 and influenza A/H3N2 viruses in the swine population. Monitoring of the circulation of influenza A viruses in the swine population is necessary for timely anti-epidemic and anti-epizootic measures to prevent epidemics, pandemics, and epizootics.
Aim: Since epidemic seasons 2017/2019, detection of Influenza C virus have been included into sentinel virological surveillance as no systematic study have been available in the Czech Republic.

Method: The sentinel surveillance of acute respiratory infections (ARIs) requires collecting of swabs (20-50 per week). Influenza A/B and panel of respiratory viruses are routinely tested so all negative sentinel samples diagnosed as acute upper respiratory infection (J00, J02, J04, J05, J06) and influenza like illness - ILI (J10.1, J10.8, J11.1, J11.8) were tested for presence of influenza C virus.

Results: Total 279 swabs were tested. Influenza C was detected in 8 cases (out of 169, 4.73%) in the 2017/18 season, no case (out of 110) was detected in the 2018/19 season. Positive cases occurred among patients of all ages, 4 occurred among children and adolescents. Originally, 4 out of 8 positive cases were diagnosed with influenza, 3 with upper airways catarrh and the oldest patient was diagnosed with pneumonia.

Conclusions: The results suggest that the circulation of influenza C may not always be demonstrable in the respective season. Influenza C virus has been regarded as a pathogen causing mild or unapparent disease. Its capture may be covered by another prevalent pathogen. Although primary infections occur primarily in childhood, detection of the virus in the elderly is not unique. Diagnosis of influenza C has provided more detailed information in ARI surveillance.

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Aim: The aim of this study was to determine the rapid and reliable detection of the viruses that may cause acute exacerbations in chronic obstructive pulmonary disease (COPD) by using the multiplex polymerase chain reaction (PCR) method.

Method: The study included 90 patients who were admitted to Emergency and Chest Diseases Clinics, between 14 January 2016 and 31 May 2017. A commercial kit was used for the isolation of viral nucleic acid from the sputum. Multiplex RNA/DNA virus master kit and specific primer-probe mixtures was used for amplification.

Results: In 7.8% of the samples, a viral agent was detected. The detected viruses were influenza A(H1N1), human parainfluenzavirus-3, respiratory syncytial virus (RSV) and rhinovirus (RV). It was observed that virus detected patients were mostly COPD stage B and stage D patients. However, there was no statistically significant difference between COPD stages in terms of virus positivity. A total of 20 patients (22.2%) had positive bacterial cultures, with the most commonly found bacteria being Streptococcus pneumoniae and Pseudomonas aeruginosa. In our study, both virus and bacteria were identified in four patients. Co-infection by RSV and Streptococcus pneumoniae was found in one patient, RV and Streptococcus pneumoniae in two patients and RV and Haemophilus influenzae in one patient.

Conclusions: In our study, RV was the most detected type. In previous studies, RV has been shown in acute exacerbations of COPD. The determination of viruses that causes exacerbations would prevent such exacerbations, and by making early detection and rapid treatment, it would yield an improve in patient’s life quality and a decrease in the demand for hospital stays.
RESULTS FROM SENTINEL SURVEILLANCE SYSTEM

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Aim: “Respiratory syncytial virus (RSV) is one of the major causes of respiratory infections during infancy with high rates of hospitalization and mortality. The RSV infection is severe also in older adults and long-term hospitalized patients.”

Method: “The current sentinel ARI (acute respiratory infection) virological surveillance requires collecting of 20-50 swabs per week according to the following criteria: acute upper airways infection (J00, J02, J04, J05, J06) and influenza (J10.1, J10.8, J11.1, J11.8). RSV A/B is detected using multiplex RT-qPCR assay.”

Results: “A total of 1,143 swabs from patients of all age groups were examined. We detected 25 RSV (out of 650; 3.8%) and 38 RSV (out of 493; 7.7%) during the seasons 2017/2018 and 2018/2019. The most affected age group were children under 5 years (18/25; 72% and 17/38; 45%). During the season 2017/2018, RSV A dominated over RSV B. RSV A/B occurred in men in 64% (16/25) whereas in women in 36% (9/25). During the season 2018/2019 (as of March 21st, 2019), occurrence of RSV A and B was balanced. RSV A/B occurred in men in 34% (13/38), whereas in woman in 66% (25/38).”

Conclusions: “Although the RSV diagnosis is part of the standard virological surveillance in the Czech Republic, more specific and detailed targeting on RSV is needed. Information obtained from such surveillance will contribute to understanding of outbreaks, clinical symptoms across age groups, epidemiological impact, and demographic data.

Supported by MH CZ - DRO (National Institute of Public Health - NIPH, IN 75010330).”
[P006] RHINOVIRUSES IN HOSPITALIZED CHILDREN WITH ACUTE RESPIRATORY INFECTION, CROATIA 2017-2019

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Aim: To determine the clinical characteristics and epidemiology of rhinovirus infection in hospitalized children with acute respiratory infection (ARI).

Method: A prospective study conducted from March 2017 to February 2019, included 427 children with ARI, admitted at Children’s hospital Zagreb. Nasopharyngeal swabs were tested for respiratory viruses by multiplex PCR and cDNA synthesis in one-step reaction, followed by detection of PCR amplicons using microchip electrophoresis.

Results: There were 259 boys and 168 girls. According to the age, the following groups were defined: 0-12 months (n=129), 13-36 months (n=117), 37-60 months (n=51), and >60 months (n=130) of age. According to the localization of infection, patients were categorized as those presented with upper respiratory tract infection (n=221), and those with lower respiratory tract infection (LRTI) (n=206). The viral etiology was proved in 74.9% of the patients. The most commonly detected respiratory virus was rhinovirus, diagnosed in 40.5% of all patients; 63.6% as monoinfection, and 36.4% as codetection with other respiratory viruses. Fifty-one percent of children with rhinovirus monoinfection presented with LRTI. There were no statistically difference in rhinovirus prevalence according to the gender, age, and localization of infection (P > 0.05). Peak incidence of rhinoviruses was registered in spring and autumn months.

Conclusions: Rhinoviruses were the most prevalent respiratory viruses in this study causing significant proportion of LRTIs. These results highlight its role in etiopathogenesis of LRTI in children of all ages.

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DETECTION OF VIRAL AND BACTERIAL AGENTS IN CHILDREN WITH LOWER RESPIRATORY TRACT INFECTION IN PEDIATRIC INTENSIVE CARE UNIT

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Aim: Lower respiratory tract infections (LRTI) are the third leading cause of death worldwide. Lower respiratory tract infection may be particularly severe in infants and children. The aim of this study was to investigate the causative agents in patients with lower respiratory tract infections such as bronchitis, bronchiolitis and pneumonia in the pediatric intensive care unit (PICU).

Method: Respiratory tract specimens (37 BAL, 23 sputum) were collected from 60 patients with LRTI in PICU. Of the patients, 40 (66.7%) were male and 20 (33.3%) were female. The age range of patients is between 40 days to 180 months (median: 24 months, mean: 40.3±4.6 months). All specimens were tested by respiratory panel.

Results: Of the 60 specimens tested, 43 (71.7%) were positive for one or more causative agents, 16 (26.7%) were negative and one specimen (1.7%) was invalid. In the 43 positive specimens, 21 (35.0%) were single (n=13, 21.7%) and dual (n=8, 13.3%) virus infections, 2 (0.2%) were single bacterial infections, and 20 (33.3%) were mix virus and bacterial infections.

Conclusions: At least one respiratory tract agent was found in 72% of patients admitted to the PICU with a diagnosis of LRTI. The most common cause of LRTI among the viruses was Human Rhinovirus, Respiratory syncytial virus (RSV), Adenovirus, and the bacteria were Haemophilus influenzae, Streptococcus pneumoniae and Morexlla catarrhalis, respectively. The most prevalent causative agents in mix infections were RSV and H. influenzae. Identification of potential causative agents in a short time is very important for the diagnosis of pneumonia. Respiratory panel* is rapid system, with results available in just over one hour.

* FilmArray LRTI Panel (BIOFIRE, Salt Lake City, UT)
[P010] CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF HUMAN PARAINFLUENZA VIRUSES BY ANALYSIS OF DATA FROM LAST 2 SEASONS AT NATIONAL INFLUENZA CENTRE SLOVENIA

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Aim: Human parainfluenza viruses (hPIVs) often cause acute respiratory infections (ARI) in children. Less is known about them in adults. At National Influenza Centre Slovenia clinical and epidemiological characteristics of hPIVs were estimated.

Method: Between weeks 40/2017-15/2019, 4893 nasal/throat swabs from patients with ARI, their demographic, clinical data were collected from 50 primary healthcare clinics and 2 hospitals. Nucleic acids were extracted; multiplex-RT-RT-PCRs were used to detect influenza and other respiratory viruses, including hPIVs (types 1-4). Age groups (AGs) were formed: <1, 1-3, 4-7, 8-14, 15-19, 20-64, ≥65 years of age (YA).

Results: HPIVs were detected in 253 (5%) specimens. Majority in young children (1-3YA, 36%) and elderly (≥65YA, 20%). Lower rates in infants (<1YA, 16%), adults (20-64YA, 14%), children (4-7, 8-14, 15-19YA with 8%, 5%, 1%). Most patients were examined in hospitals (69%-96%), except AG 15-19YA (33%).

Majority of patients reported cough (90%-93%) and fever (55%-81%). Breathing difficulties were frequent in all AGs (15%-40%), but significantly more in patients ≥65YA (70%). Bronchiolitis was frequent in infants (<1YA, 23%), while pneumonia was frequent in adults (20-64YA, 20%). In patients ≥65YA bronchiolitis and pneumonia occurred at higher rates (22%, 24%). Acute respiratory distress occurred only in elderly (2%).

HPIVs were detected through seasons, except in July, August. Majority of ARI were caused by hPIV-3 (40%), hPIV-1 (33%), followed by hPIV-2 (15%), hPIV-4 (12%). HPIV types co-circulated; no circulation pattern was observed.

Conclusions: Data showed higher disease burden of hPIVs in young children than in infants, older children, adults. But significant disease burden was observed in elderly.
INVESTIGATION THE PREVELANCE AND SEASONAL ANALYSIS OF HUMAN PARAINFLUENZA VIRUSES (HPIVS) IN PEDIATRIC PATIENTS

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Aim: HPIVs cause the majority of children acute respiratory tract infections and lead to morbidity and mortality worldwide. The aim of our study is to investigate the prevalence and seasonal analysis of HPIVs in pediatric patients with respiratory tract infection who are enrolled to our hospital

Method: A total 396 patients hospitalized in Gazi University Hospital between, March 2016 to 2019 were included in the study. Viral nucleic acids were extracted with Isolation Kit. Multiplex Real Time-PCR was performed using Respiratory Pathogens kits.

Results: Three hundred sixty-nine patients 213(52,7) male and 156(42,3) female, between 1 months and 16 years were included to the study. There was no significant relationship between age, gender and HPIVs positivity. Total HPIVs positivity rate was found as 7,3(27/396). The distribution of HPIVs positivity rates respectively %29,6(8/27) for pediatric outpatients, %14,8(4/27) for pediatric infection, %14,8(4/27) for pediatric hemotology, %11,1(3/27) for pediatric bone marrow unit, %7,4(2/27) for pediatric oncology, %7,4(2/27) for pediatric health, %7,4(2/27) for pediatric intensive care unit, %7,4(2/27) newborn intensive care unit. The distribution of HPIVs positivity rates respectively %29,6(8/27) for HPIV4, %22,2(6/27) for HPIV3, %18,5(5/27) for HPIV2 and HPIV3 together, %14,8(4/27) for HPIV2, %11,1(3/27) for HPIV1, %3,7(1/27) for HPIV3 and HPIV4 together. The distribution of HPIVs positivity rates respectively the most common %16,2(6/27) in October, %18,5(5/27) in December %18,5(5/27) in November.

Conclusions: HPIVs was most commonly seen in autumn and winter seasons. In this study, it was shown that the occurrence of this virus in childhood was frequently and HPIVs was seen most in pediatric outcomes and immunocompromised children. Therefore, HPIVs should be considered in especially immunocompromised pediatric patients with viral upper respiratory tract infections on autumn and winter season.
Aim: Respiratory syncytial virus (RSV) is a major cause of acute bronchiolitis and pneumonia in infants and young children worldwide. By 2 years of age virtually all children have been infected with RSV. However, the disease is self-limited in most. We aimed to evaluate the seasonal relationship of RSV positivity in pediatric patients.

Method: The results of 416 patients, 171 (41.1%) female and 245 (58.8%) male, aged between 0 and 17, with pneumonia, acute bronchitis, respiratory distress syndrome and other diseases who were hospitalized between February 2014 and February 2019 were evaluated retrospectively. The nasopharyngeal specimens were studied by Real Time-PCR.

Results: The total number of positive RSV was found 49/416 (11.7%). The distribution of the positivity samples by age were 2 (0.48%), 24 (5.7%), 13 (3.1%), 6 (1.4%), 2 (0.48%): 0-1-month, 1 month-1 age, 1-5, 5-12 and 12-18 age groups, respectively. The distribution of RSV positive sample’s numbers was 24 (5.7%), 11 (2.6%), 6 (1.4%), 5 (1.2%), 2 (0.48%), 1 (0.2%); pediatric infection clinic, intensive care unit, hematology clinic, oncology clinic, newborn intensive care unit and newborn clinic respectively. The most frequent occurrence during the year is winter and the most positive occurrence was seen in January. In the winter 40 (9.6%), in the spring 7 (1.6%), in autumn 2 (0.48%) patients were positive.

Conclusions: RSV is more frequent and serious in infants and immunosuppressive patients. In addition, the frequency of RSV increases seasonally during the winter months. Definitive treatment of RSV and vaccination is not available; therefore, prevention is the actual solution. Hand washing, cleaning environmental surfaces, avoiding overcrowded places, isolating people with infection is very important for preventing transmission of the virus on this season, especially among immunocompromise patients.
[P013] LOW FREQUENCY OF REDUCED NEURAMINIDASE INHIBITOR SUSCEPTIBILITY IN TWELVE EU/EEA COUNTRIES, 2008-2018

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12.9. Norwegian Institute of Public Health, Department of Influenza, Nic Norway, Oslo, Norway
13 National Reference Laboratory for Influenza and Other Respiratory Viruses, Infectious Diseases Department, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
14.10. National Influenza Reference Laboratory, Infectious Diseases Department, National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal
15.11. National Influenza Center, “Cantacuzino” National Medico-Military Institute for Research and Development, Bucharest, Romania
17. Instituto de Salud Carlos III, Madrid, Spain
18. The Public Health Agency of Sweden, Unit for Laboratory Surveillance of Viral Pathogens and Vaccine Preventable Diseases, Solna, Sweden
19.14. Unit for Laboratory Surveillance of Viral Pathogens and Vaccine Preventable Diseases, The Public Health Agency of Sweden, Solna, Sweden
20. Public Health England, Colindale, United Kingdom
21. Public Health England, Respiratory Disease Department, London, United Kingdom
22. Rivm, Bilthoven, Netherlands

Aim: Neuraminidase inhibitors (NAIs) are used as prophylaxis and treatment against influenza. We analysed the frequency of reduced (RI) or highly reduced (HRI) NAI susceptibility in specimens received through influenza surveillance in the European Union/European Economic Area (EU/EEA) countries in order to inform the treatment guidelines.

Method: Pheno- and genotypic RI/HRI to oseltamivir and zanamivir in 12 EU/EEA countries who submitted influenza antiviral susceptibility data to The European Surveillance System during
2008/09-2017/18 were included. Comparison of means was performed by Fisher’s exact test for categorical variables (significance, p<0.05).

Results: The overall prevalence of RI/HRI to NAIs was 1.0% in the 27371 analysed influenza viruses. The frequency of RI/HRI varied by season, (sub)type, country, hospitalisation and treatment status, while vaccination status did not affect the susceptibility to NAIs. The highest frequency of 74% was detected in 2008/09 for A(H1N1) former seasonal viruses. A(H1N1) pdm09 viruses showed 1.3%, A(H3N2) 0.2% and B viruses 0.2% RI/HRI to oseltamivir and 0%, 0.1%, 0.2% and 0.2% to zanamivir, respectively. Across the seasons, RI/HRI of A(H1N1) pdm09 for oseltamivir was highest in France (3.7%, p<0.0001). Of the hospitalised patients, 108 (2.9%; p<0.0001) had a virus showing oseltamivir RI/HRI compared with the 37 (0.5%) of the outpatients. Of the 177 patients with known treatment status with RI/HRI to oseltamivir, 63 (36%; p<0.0001 vs treated) had not been treated with NAIs.

Conclusions: Low frequency of reduced NA susceptibility supports the use of NAIs against severe influenza. Continuous monitoring of susceptibility is crucial in patients treated and not treated with NAIs.
TRANSVERSE MYELITIS ASSOCIATED WITH ENTEROVIRUS D 68 INFECTION

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Aim: “A previously healthy four year old girl admitted to Ankara Training and Research Hospital with the complaints of neck and right arm pain, loss of strength and sense in the right arm, unable to hold neck upright, facial asymmetry, running nose and cough was diagnosed as Enterovirus D68 infection with multiplex PCR.”

Method: “Firstly, nasopharyngeal swab sample of the patient was positive for M.catarrhalis and S.pneumoniae by multiplex PCR. The test was repeated with the deep tracheal aspirate sample and Enterovirus was found to be positive. Isolation of samples was done by magnetic bead extraction*

Results: “Isolated DNAs were examined by Real-Time PCR method using two devices** and the results were evaluated qualitatively. Subtype of the Enterovirus was detected as Enterovirus D68 with specific probes in the same sample and in recently collected tracheal aspiration samples in the Virology Laboratory of General Directorate of Public Health for confirmation. In recent years, clinical and laboratory features of similar patients have been presented as an increasingly common infection worldwide. Enterovirus D68 should be persistently investigated in such cases, since it is increasing in prevalence, can make outbreaks, and cause severe respiratory tract infections and / or neurological symptoms.”

*Montania 16 (Anatolia Geneworks®, Turkey).
**Montania 4896 (Anatolia Geneworks®, Turkey) and Bosphore® Viral Meningitidis Panel Kit v1 (Anatolia Geneworks®, Turkey)
Aim: “To isolate and identify the human metapneumonia virus (HMPV) from hospitalized children with acute respiratory infection (ARI) in pseudostratified human airway epithelium (HAE) cell culture and character its replication and infection.”

Method: “Well-differentiated pseudostratified mucociliary HAE cells grown at the air–liquid interface was used to isolate the HMPV strains from positive nasopharyngeal aspirate specimens of hospitalized children with ARI in Beijing. The HMPV cell tropism and cytopathic effect were detected by immunostaining staining with anti-β-tubulin and anti-ZO1 antibodies. The propagation and replication kinetics of HMPV on three-dimensional (3D) cells was compared with those of other cell lines by real-time PCR assay. Additionally, the morphology of HMPV virions was observed by electron microscopy.”

Results: “HMPV could be isolated from clinical sample and infect both ciliated and non-ciliated cells within the 3D HAE cell culture, with reduction of cilia and airway epithelial slight damage. For LLC-MK2 and Vero-E6 cells, HMPV proliferated from the first day post infection at a uniform rate and peaks on the fourth and sixth day, respectively. In contrast, HMPV did not propagate within the first three days, but then began to proliferate rapidly and peaked on the sixth day. HMPV is a kind of irregular spherical particle with envelope and spike, diameter between 150-600nm observed under transmission electron microscopy.”

Conclusions: “3D HAE cells can be used to isolate HMPV from clinical samples. Compared with the LLC-MK2 and Vero-E6 cells, the HMPV replication kinetics with 3D HAE cells differed from traditional cells.”
[P016] EVALUATION OF THE RESPIRATORY VIRAL AETIOLOGY BY MULTIPLEX REAL-TIME PCR IN PATIENTS WITH UPPER RESPIRATORY TRACT INFECTIONS IN ANKARA TRAINING AND RESEARCH HOSPITAL

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Objective: The aim of this study was to evaluate the viral respiratory tract infection agents in our hospital by using molecular diagnostic techniques.

Method: One hundred forty one patients with the symptoms of upper respiratory tract infections were included in the study. The study was carried out in the Molecular Microbiology Laboratory of Medical Microbiology Department of the Ankara Training and Research Hospital between October 2018 and March 2019. Nasopharyngeal smears and deep tracheal aspirates were analyzed. Extraction and isolation of the viral nucleic acid was performed by device*, multiplex Real time PCR amplification was performed by device**.

Results: Of the 141 patients, 41(%29,08) were adults and 100(%70,92) were children. The most frequently detected viruses according to age groups were as follows; 16 RSV (59.2%) in 0-12 months; 15 rhinovirus (44.1%) in 1-5 years; 2 rhinovirus (5.8%) in 6-18 years and 15 influenza A(1.4%) in 18 years and older ages. Among 55,31 % of the 141 patients, a single viral agent was detected in the etiology and multiple agents were detected in 16,31% of 141 patients.

Conclusion: Viruses causing respiratory tract infections are a major cause of mortality and morbidity. The determination of respiratory viruses is very important. We conclude that analysis by multiplex PCR in symptomatic patients will be beneficial directly for the patients and indirectly for the national economy.

* Magnesia 16 (Anatolia Geneworks®, Turkey)
**Bosphore Respiratory Pathogens Panel Kit (Anatolia Geneworks®, Turkey) in Montania 4896 (Anatolia Geneworks®, Turkey)
CONTRIBUTION OF RESPIRATORY Syncytial Virus (RSV) AMONG PATIENTS <15 YEARS HOSPITALIZED WITH SEVERE ACUTE RESPIRATORY INFECTION (SARI) IN MILAN, 2014-2017

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Aim: Respiratory syncytial virus (RSV) infections may range from cold to severe acute respiratory infection (SARI) and are responsible for substantial pediatric morbidity. We describe the results of RSV molecular detection in respiratory samples collected from children <15 years hospitalized with SARI in Milan (Italy) during four consecutive years.

Method: From January 1st, 2014, to December 31st, 2017, 3013 respiratory samples (2826 upper-respiratory-tract [URT] and 187 lower-respiratory-tract [LTR] specimens) collected from as many children <15 years hospitalized with SARI at an University hospital in Milan were analysed. After DNA/RNA extraction, samples were tested by a multiplex real-time PCR to detect RSV and other respiratory viruses.

Results: 571 (19%) respiratory samples tested RSV-positive. RSV-positivity rate by sample type was similar (URT vs LRT: 19.2% vs 14.4%; p=0.09). The median age of RSV-positive cases was 6.6 months (inter-quartile range: 17.2 months); 52.2% were males. 62.2% (355/571) of RSV-positive samples were identified in children <1 year and 12.4% (71/571) in those <1 month. RSV was detected throughout the study period; 59.2% (338/571) cases were identified during seasonal peaks (December-February). In 49.9% (285/571) of RSV-positive samples at least another virus (mainly Rhinovirus: 45.9%) was detected, particularly (60%; 171/285) in samples collected from children >1 year.

Conclusions: Accordingly, to other studies, RSV was detected in 19% of hospitalized-SARI cases <15 years, mainly in children <1 year and in December-February. Sampling of upper or lower airways resulted in similar RSV-positivity rate. Routine molecular testing to detect RSV is warranted to improve clinical management of pediatric patients.
[P018] CLINICAL AND EPIDEMIOLOGICAL IMPACT OF HUMAN PARAINFLUENZA VIRUSES INFECTIONS IN IMMUNOCOMPETENT AND IMMUNOCOMPROMISED PATIENTS.

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Aim: Respiratory infections due to human parainfluenza viruses (hPIVs) are widely underestimated in Italy. HPIVs cause also severe syndromes and they circulate in all seasons of the year. The aim of present study was to investigate the epidemiology of hPIVs infections and their clinical manifestation in a hospital-based population.

Method: A retrospective study of the circulation of different HPIV genotypes was performed analyzing all respiratory samples positive for HPIVs in the period August 2016 to August 2018. Respiratory samples were routinely tested with a panel of in-house real-time RT-PCR for detection and quantification of respiratory viruses, including HPIVs 1-4.

Results: A total of 104 (2.4% of a total) patients resulted positive for hPIVs. Of them, 67/104 (64.5%) had signs and symptoms of URTI, while 37/104 (36.5%) had signs and symptoms of LRTI. The most frequently detected genotype was hPIV-3 (96/104, 92.3%) followed by hPIV-4 (7/104, 6.1%), and hPIV-2 (1/104, 0.9%), while no cases of hPIV-1 were observed. Three outbreaks of HPIV-3 were observed during the study period in onco-hematological ward. Prolonged hPIV shedding (>21 days) was observed in 8 immunocompromised patients.

Conclusions: In this study hPIV infection is a considerable factor of morbidity in adults as well as in children. hPIV-3 was the most representative and widespread genotype, with a serious impact as cause of intra-hospital outbreak. The introduction of multiplexed molecular panel for the diagnosis of respiratory viruses have raised the attention on the role of non-influenza viruses as a cause of severe and/or prolonged infections.
[P019] EXTENSIVE HOSPITAL CLUSTERING REVEALED BY MOLECULAR CHARACTERIZATION OF INFLUENZA A VIRUS INFECTION.

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Aim: To describe the characteristics of hospitalized patients with influenza A virus (InfA) infection and to investigate transmission dynamics within a 1900-bed, acute-care, teaching hospital.

Method: During season 2016-17, all hospitalized patients >18 years old with laboratory-verified (real-time PCR) InfA were identified. Cases were characterized according to age, sex, co-morbidity (Charlson score), hospital ward, antiviral therapy, viral load, length of hospital stay (LOS) and 30-day mortality. Univariate survival analysis was performed using log rank test and multivariate models by Cox regression. Samples from patients involved in outbreaks were chosen for sequence analysis and the web-based INSaFlu-tool1 was used for bioinformatics. In order to detect clustering, phylogenetic trees were constructed using concurrently circulating InfA strains from patients in the community as background.

Results: Of 435 InfA cases, 114 (26.2%) were classified as health-care-associated infections (HCAI). The overall 30-day mortality rate was higher in the HCAI-group (9.6% compared with 4.6%), p <0.05. Median LOS after sampling was 6 and 10 days respectively for surviving non-HCAI and HCAI cases. In total, 242 (56%) patients received antiviral therapy. A phylogenetic tree based on the HA-gene identified 8 hospital clusters (with ≥3 cases), related in time (sampled ≤ 7 days) and location (shared ward), supporting a close relationship between outbreak cases.

Conclusions: Frequent in-hospital transmission of InfA virus was revealed by a molecular, clinical and epidemiological investigation. This is useful for investigating hospital outbreaks and may support evaluation of preventive measures for nosocomial influenza.
[P020] CHARACTERISTICS OF HUMAN MONOCLONAL ANTIBODIES TARGETING MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS SPIKE PROTEIN IN HUMAN DPP4 KNOCK-IN MOUSE MODEL

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Aim: “Middle East Respiratory Syndrome coronavirus (MERS-CoV) is a zoonotic emerging virus causing severe pneumonia and 35% high mortality. However, there are no antivirals for MERS infection approved by FDA. To respond MERS outbreak rapidly, development of neutralizing antibodies against MERS-CoV has been required for safe and effective MERS treatment. Thus, we generated human monoclonal antibodies (mAbs) from Korean MERS patients’ B cells and analyzed the characteristics of these antibodies in vitro and in vivo human DPP4 knock-in mouse model.”

Method: “To evaluate the antibody affinity to MERS-CoV Spike (S) protein, ELISA was performed. PRNT with using MERS-CoV was undergone to estimate neutralizing activity of our mAbs. To validate the therapeutic or prophylactic efficacy of these antibodies, human DPP4 knock-in mice were infected with MERS-CoV in both therapeutic and prophylactic settings.”

Results: “Eleven antibodies had high affinity to S protein. Among them, eight antibodies showed neutralizing activity against MERS-CoV infection. 90-F1 antibody exhibited the most effective neutralizing activity as IC₅₀ 0.006 ug/mL by binding to RBD. This antibody showed high efficacy in protection from MERS-CoV infection, with reduction of viral titers in lungs and brains, as well as 100% survival at low antibody concentration in vivo mouse model.”

Conclusions: “These results show that our antibodies can be used to detect MERS-CoV as a diagnosis tool owing to their high affinity to S protein. Especially, 90-F1 can be applied for prophylaxis or for the treatment of human MERS-CoV infection.”
RESPIRATORY SYNCYTIAL VIRUS GENOTYPES CIRCULATING IN IZMIR, TURKEY, IN FIVE CONSECUTIVE YEARS

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Aim: This study is aimed to evaluate RSV genotypes circulating in our region between 2014 and 2018.

Method: The study was conducted with samples collected between January 2014 and October 2018 at a university hospital in Izmir, Turkey. Samples of patients with a presumed viral respiratory infection were analyzed by a multiplex PCR assay* or five-tube multiplex**. RSV positive samples as mono-infection were re-evaluated by an in-house RT-qPCR for RSV type and Ct (cycle threshold) value determination. Samples with Ct<30 were chosen for sequencing targeting RSV G-protein and using ABI Prism 3130 Genetic Analyser (Applied Biosystems).

Results: RSV was detected in %13 of the samples (336/2583). RSV-B infections predominated in 2015, while RSV A was dominant or in similar numbers with RSV-B in the other years. Among 236 samples, 145 (77 RSV-A and 68 RSV B) were sequenced and satisfactory results were obtained from 53 RSV-A and 37 RSV-B samples. All RSV A samples were identified as ON1 genotype. RSV B samples belonged to two genotypes, BA09 (n:8, 22%) and BA10 (n: 29, 78%). Genotypes ON1 and BA10 were detected in each year of the study, while BA09 was detected in 2017 and 2018 (Table 1).

Conclusions: ON1, which is the dominant phenotype in Europe and Turkey since 2012, was identified as the only RSV-A genotype. RSV-B samples belonged to two genotypes (BA09 and BA10). BA09 was present in the last two years of the study.

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** Fast Track Diagnostics/Respiratory Pathogens-21 (Junglinster, Luxemburg)
Aim: “To determine the viral etiology of respiratory infections in hospitalized children with severe acute respiratory infection (SARI) and investigate the epidemiology and clinical profiles of the viruses.”

Method: “1003 nasopharyngeal aspirates from children with SARI in Shanghai 2013-2015 were screened for 15 respiratory viruses using multiplex nested RT-PCR assay for common respiratory viruses. All amplicons were confirmed by sequencing and the clinical and epidemiological characteristics of the patients with respiratory virus infection were analyzed.”

Results: “viral pathogens were identified in 603 children (60.12%) and 36.53% of patients detected positive for more than one virus. The most frequently tested pathogens were human rhinovirus (HRV) (27.92%), adenoviruses (ADV) (16.85%), and human bocavirus (HBoV) (14.06%) and human respiratory syncytial virus (hRSV) (10.27%). Influenza viruses (Flu) and hRSV infections mainly occurred in winter, enterovirus (ENV) infection occurred in summer, HRV detections peaked in autumn, whereas ADV and HBoV predominant in summer and autumn 2014, respectively. A higher detection frequency of HRV occurred in the <4, ADV in the 1-4 and hRSV in <1 years age groups, but there no significant differences in age group distribution for other detected viruses. Cough and gasping in hRSV-infected patients and diarrhea in HCoVs-infected patients were observed higher than in the other cases.”

Conclusions: “Our study provided the circulation pattern of 15 respiratory viruses in Shanghai and reported of the potential impact of four viruses (HRV, ADV, HBoV and hRSV) as causes of SARI in hospitalized children.”
Aim: This pilot study evaluated the adequacy of self-collected oropharyngeal swabs compared to those collected by trained physicians for molecular detection of respiratory viruses.

Method: Oropharyngeal swabs were collected from influenza like-illness cases in Lombardy during the 2018/2019 season. Two groups of samples were considered: group 1) 131 swabs collected by general practitioners operating within the Italian Influenza Surveillance Network; group 2) 131 swabs self-collected by hospital healthcare workers (doctors, nurses, technicians, in-training students) after being trained on the sampling procedure by both an explanatory brochure describing the steps of swab collection, point-by-point, and a telephone call to a study staff member who guided sample collection. RNA was extracted from each swab and tested for the detection of the human ribonuclease P gene (RNP) by real-time RT-PCR. Samples with a cycle threshold (Ct)<35 were considered adequate for further virological analysis. Respiratory syncytial virus (RSV) was detected by real-time RT-PCR.

Results: All samples were positive to RNP detection with Ct<35. The mean Ct value was 25.46 (SD: ±2.40; range: 18.65-30.10) for swabs collected by physicians and 25.93 (SD: ±2.22; range: 19.48-33.13) for self-collected swabs, with no statistically significant difference (p=0.10). RSV-positivity rates were similar among both groups (10.9% vs 7.3%; p=0.39).

Conclusions: Self-collected oropharyngeal swabs resulted adequate and comparable to those collected by trained physicians for molecular detection of respiratory viruses. Self-sampling can be a worthwhile strategy of sample collection to implement molecular surveillance of respiratory viruses or to evaluate vaccine effectiveness involving population at lower costs.

1SIGMA VIROCULT (MW951S), MWE.
COMPARISON OF 3 POINT-OF-CARE TESTING METHODS FOR THE DETECTION OF INFLUENZA AND OTHER RESPIRATORY PATHOGENS

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Aim: to assess the utility of fast Syndromic and single FluA/B testing strategy and compare Alere™ i Influenza A & B and, QIAstat-Dx Respiratory Panel with BioFire RP2plus used in the lab as routine method.

Method: 95 nasopharyngeal fresh samples collected in wards of the University Hospital of Crete during respiratory season 2019 were tested with all 3 methods. Laboratory’s method BioFire RP2 plus using a FilmArray 2.0 system was compared with QIAstat-Dx RP using QIAstat-Dx Analyzer and Alere™ i Influenza A & B using ID NOW™.

Results: In respect to influenza virus, out of 95 samples there were 32 positive—all influenza A type— and 63 influenza negative samples as well as 2 Flu A equivocal with BioFire RP2plus. Of them, QIAstat-Dx RP reported 29 positive and 61 negative for influenza samples while 5 tests failed and not retest was performed. Alere i reported 28 positives and 67 negatives. Among influenza negative samples, 36 respiratory targets were detected in 22 patient samples using FilmArray RP2 plus and 4 influenza A co-infections. For QIAstat Dxp, there were 22 total detections in 20 samples and 2 co-infections.

Conclusions: Syndromic panels detected pathogens in 35% of Influenza negative samples supporting patient management. QIAstat Dxp produced 78% agreement in comparison with FA RP2 plus while demonstrating a 5.2 % test failure rate. Alere I A/B provides fast results with reasonable agreement compare to the lab method and could be consider as testing alternative mainly for outpatients without risk factors.
DEMOGRAPHICAL AND CLINICO-BIOLOGICAL CHARACTERISTICS OF RSVA AND RSVB INFECTIONS, COMPARISON OF TWO INTRA EPIDEMIC PERIODS IN 2011 AND IN 2018

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**Aim:** the aim of the study was to compare demographical and clinical characteristics between RSVA and RSVB infections among patients admitted in the Toulouse University Hospital.

**Method:** the first 100 patients RSV positive in January 2011 and in January 2018 were included in the study. Patients suffering from respiratory viral co-infections were discarded. Respiratory samples had been tested by molecular assay*. Demographical and clinico-biological data were collected from the computerized patient record.

**Results:** RSVA represented 62% in 2011, 52% in 2018 (p=ns). The sex ratios (M/F) were 1.4 for RSVA - B in 2011 and 0.73 in 2018 (p=0.02). Mean age was 4.01 (±13.58) year-old in 2011 and 34.34 (±33.93) in 2018 (p<0.0001), similar for both RSV types. Risk factors and/or co-morbidities were present in 43.5% in 2011 and 81% in 2018 (p<0.0001), without any difference between RSVA and B. Exposition to tobacco smoke was the most prevalent risk (15.2%) among infants under 2yo, whereas it was haematological malignancies in adults (7.5%). Four fatal evolutions concerned adults suffering from pre-existing chronic pathology: 3 in 2011 (2 RSVA) and 1 (RSVA) in 2018.

**Conclusions:** RSVA and RSVB co-circulated in January 2011 and 2018 and did not seem to have a specific pathogenicity. Adults can be particularly targeted. We highlighted risk-factors that have to be taken into account in order to prevent RSV infection.

*(2011: Respifinder Kit, Pathofinder®; 2018: Allplex respiratory full panel, Seegene®)*
IMPLEMENTATION OF COBAS LIAT INFLUENZA A/B AND RSV ASSAY INTO ROUTINE OF A SMALL THROUGHPUT MOLECULAR CLINICAL LABORATORY

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Aim: “Fast and accurate identification of influenza and RSV is important for successful clinical practice, infection control and use of antibiotics. Herein we report the usefulness of compact PCR system * introduced in clinical laboratory.”

Method: “During 2018-2019 flu season, 1824 respiratory swabs were tested for Influenza A/B (FluA/B) and RSV in our laboratory. Assay** (reference method) and multiple PCR system*** are routinely used. We detected FluA and RSV in 433 and 114 tested samples, respectively. To evaluate system*, a comparison with the reference method and test**** was carried out using 78 swabs. Instead of universal transport medium, swabs are transported in sterile saline solution.”

Results: “In 78 samples tested system* produced four invalid results (5.13%). Similar rate of invalid results has already been reported in previous studies. Comparison of system* against reference method showed 100% (23/23) specificity for FluA/B and 95.8% for RSV (22/23). Sensitivity was 96.4% (26/27) for FluA and 80 % for FluB (6/8) whereas RSV reached 100% Test**** was used on selected 40 of 78 samples and concurred with the reference method resulting in 100% sensitivity and specificity for FluA and RSV. No invalid results were reported. Implementation of system* in routine work contributed to shorter turn-around time for influenza diagnosis with 79.6% of test results completed within the same working day, compared to 61.3% with the reference method.”

Conclusions: “It was shown that system* produces reliable results available within shorter time for influenza diagnosis and is useful in clinical laboratory.”

* cobas Liat System
** LightMix Modular
*** FilmArray
**** Xpert Xpress Flu/RSV
[P027] MOLECULAR TYPING OF HUMAN PARECHOVIRUS IN RESPIRATORY SAMPLES IN BELGIUM

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²Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium

Aim: “Human parechoviruses (HPeV) usually cause mild respiratory or gastrointestinal symptoms, but can occasionally cause serious illness in infants. For epidemiological surveillance, HPeV-positive respiratory samples identified at the University Hospitals of Leuven were molecularly typed in the context of our reference laboratory activities for respiratory pathogens in Belgium.”

Method: “A molecular diagnostic test for HPeV is included in the in-house respiratory panel for simultaneous detection of 29 different respiratory pathogens, used as part of the routine clinical practice at the University Hospitals of Leuven. For HPeV-positive respiratory samples, the VP3/VP1 junction was amplified by nested PCR and sequenced, and HPeV types were identified by BLAST. Typing was confirmed by phylogenetic analysis.”

Results: “Twenty-four patients with HPeV-positive respiratory samples were identified from January to December 2018. Almost all samples were from pediatric patients ≤ 2 years of age. In all but two cases, co-infections with multiple other pathogens were detected. Co-infecting pathogens were Streptococcus pneumoniae (in 15 cases) enterovirus/rhinovirus (13), adenovirus (6), cytomegalovirus (5), respiratory syncytial virus, bocavirus, influenza virus, coronavirus and Pneumocystis jirovecii (each in 2 cases), and parainfluenzavirus and metapneumovirus (one case each). The HPeV type could be determined in 19 samples: 5 were HPeV1, 9 HPeV3, 2 HPeV5 and 3 HPeV6.”

Conclusions: “The presence of HPeV in respiratory samples was almost always accompanied by other respiratory pathogens, making it difficult to assess it’s etiological contribution to the respiratory infection. Different HPeV types were found to be circulating in Belgium, with a high prevalence of HPeV3.”
**Aim:** Assess performance of the immunochromatographic test *compared to a polymerase chain reaction (PCR) for influenza A/B in a hospital setting.

**Method:** Influenza test results from 2018.01.31-2019.03.31 were reviewed for ABN and Influenza PCR results performed the same day. ABN was used for patients with influenza symptoms. In total 3515 samples were received. 547 ABN results were available. Hereof, PCR results were available for 333 individual patients. The in-house RT-PCR detects influenza A and B ribonucleic acid. 1 sample was inconclusive by PCR, 2 inhibited. Rstudio was used for statistical analysis of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and confidence intervals (CI) for the ABN.

**Results:**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Influenza A</th>
<th>PCR POS</th>
<th>PCR NEG</th>
<th>TOTAL</th>
</tr>
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<tbody>
<tr>
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<th>Influenza B</th>
<th>PCR POS</th>
<th>PCR NEG</th>
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**Conclusions:** The test kit* insert reports the sensitivity for influenza A to 70-89% and influenza B to 50-69 % (1). This study finds a significant lower sensitivity in a clinical setting. The test kit* insert recommends retesting negative samples with a molecular assay (1), which our results support. A limitation of this study could be poor sample quality. The test kit* insert informs that false negative test results are more likely during influenza peak activity (1), but because of the study size, we have not differentiated the samples into peak/non peak season. With the available technology, it is possible to get fast and reliable PCR results.

**References:**
1. BinaxNOW® Influenza A & B Card (Moderate Complexity) CLSI + More Packet, IN416050 Rev. 8 2015/04
* Alere/Abbott BinaxNOW Influenza A & B Card (ABN)
EPIDEMIOLOGY OF HUMAN RESPIRATORY SYNCYTIAL VIRUS CIRCULATING IN BELGIUM BETWEEN 2011 AND 2019

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Aim: “The aim of this study was to determine the circulating Human respiratory syncytial virus (HRSV) subtypes and genotypes during eight respiratory seasons (2011 to 2019) in Belgium.”

Method: “1756 HRSV positive patient samples from University Hospitals Leuven and AZ Sint-Jan, Bruges, collected between October 2011 and March 2019. Samples were subtyped with qRT-PCR. A subset of samples was genotyped based on the second hypervariable region of the ectodomain of the G-gene, using RT-PCR and Sanger sequencing.”

Results: “The overall prevalence of both subtypes of HRSV is similar over the course of eight years, with 739 (42%) and 827 (47%) positive samples for HRSV-A and HRSV-B respectively. Thirty-nine samples (2%) were positive for both subtypes. HRSV subtypes co-circulated at comparable levels, with exception of HRSV-B dominance in the seasons 2013-2014, 2016-2017 and 2018-2019, and HRSV-A dominance in the 2014-2015 season. Preliminary data suggests that the dominant genotype of HRSV-A changed from GA2 to ON1 between season 2011-2012 and season 2012-2013. Since the 2013-2014 season, all of the genotyped HRSV-A strains contain the 72nt duplication and cluster with genotype ON1. All HRSV-B strains sampled between October 2011 and March 2019 clustered with genotype BA.”

Conclusions: “During the last six HRSV seasons in Belgium, only genotypes which contain a duplication in the second hypervariable region of the G-gene, ON1 (HRSV-A) and BA (HRSV-B) are circulating in Belgium. These genotypes have been described in literature to be currently the most prevalent strains worldwide.”
**[P030] GENETIC CHANGES OF INFLUENZA A (H1N1) PDM09 VIRUSES CIRCULATING IN 2018/2019 SEASON AFFECTED SUBTYPING OF INFLUENZA VIRUS BY REAL-TIME RT-PCR ASSAY**

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**Aim:** Reduced performance of real-time RT-PCR (rRT-PCR) for influenza A(H1N1) pdm09 virus subtyping was observed during the 2018/2019 season. We analysed and compared the primers/probe binding regions in the hemagglutinin (HA) gene to HA sequence of circulating strains.

**Method:** 713 respiratory samples collected from in/outpatients with influenza illness in Lombardy (Northern Italy) during the 2018/2019 season were analysed. Influenza viruses were typed (A/B) and subtyped (H1N1pdm09/H3N2) by rRT-PCR targeting matrix/nucleoprotein and HA gene, respectively, according to international protocols. The expected difference between Ct values obtained from A-typing and H1N1pdm09-subtyping assays \( \Delta_{H1-A} \text{Ct} \) is <5. Full-length HA gene (nt. 1-1778) sequence of A(H1N1) pdm09 circulating strains were obtained and compared to primers/probe sequences used in subtyping rRT-PCR assay.

**Results:** Influenza A viruses were detected in 390/713 (54.7%) specimens, 48.5% (189/390) were A(H1N1) pdm09. 52/189 (27.5%) A(H1N1) pdm09-positive samples showed a \( \Delta_{H1-A} \text{Ct} \geq 5 \) (range: 5.00-14.95). 24/52 A(H1N1) pdm09 with \( \Delta_{H1-A} \text{Ct} \geq 5 \) and 71/137 with \( \Delta_{H1-A} \text{Ct} < 5 \) were sequenced. Four nucleotide mismatches (C861T+C867T in the forward primer and A897G+A905C in the probe)¹ were detected in 17/24 (70.8%) HA sequences of A(H1N1) pdm09 with \( \Delta_{H1-A} \text{Ct} > 8 \). No mismatches were observed among HA sequences of A(H1N1) pdm09 with \( \Delta_{H1-A} \text{Ct} < 5 \).

**Conclusions:** Four nucleotide mismatches in primers/probe of subtyping rRT-PCR assay were observed in nearly 30% of A(H1N1) pdm09 viruses circulating in Lombardy during the 2018/2019 season. These point mutations reduced the primer/probe binding efficiency affecting the performance of rRT-PCR assay for subtyping. These results emphasised the need of continuously updating the molecular assays for influenza detection considering the constant evolution of influenza viruses.

¹HA1 numbering
A human parainfluenza virus type 3 (HPIV-3) outbreak occurred in a stem cell transplant (SCT) clinic in July 2018. To differentiate between community-acquired (non-SCT clinic) and SCT clinic outbreak-associated infections and to improve outbreak response capacity we developed a next generation sequencing (NGS) method for timely sequencing of HPIV-3 directly from clinical specimens.

**Method:** An amplicon-based NGS approach for HPIV-3 whole genome sequencing was developed. HPIV-3-laboratory confirmed respiratory specimens collected in July from 8 SCT clinic outbreak-associated patients and from 9 temporally-associated community-acquired patients were amplified. DNA libraries were constructed using Nextera XT DNA Library Prep Kit and paired-end sequencing was performed on the Illumina MiSeq. De novo and reference-guided genome assemblies were achieved using CLC Genomics Workbench V11.0. A phylogenetic tree of the genome sequence alignment was constructed by the neighbor-joining method implemented in MEGA V7.

**Results:** HPIV-3 whole genome sequences were obtained from all 8 SCT clinic patients (median age: 62 years) and 9 community-acquired patients (median age: 34 years). Phylogenetic analysis showed that sequences from the 8 outbreak-associated patients clustered together separately from the 9 community-acquired patients. HPIV-3 from all 8 SCT clinic patients had identical sequences except for one which had a silent mutation containing a degenerate base.

**Conclusions:** NGS allows discrimination of the relatedness of specimens obtained from temporally-related outbreak and community-acquired patients and can confirm a single source of transmission. NGS provides a powerful molecular and clinical tool to investigate outbreak-associated HPIV-3 infections, infection control prevention and help distinguish from community-acquired HPIV-3 infections.
[P032] WARD AND LABORATORY BASED EVALUATION OF THE QIAGEN QIASTAT MOLECULAR POCT RESPIRATORY VIRUS PANEL

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Aim: Molecular point-of-care tests (POCTs) for respiratory viruses allow the earlier detection of these viruses and the use of influenza antiviral drugs, with the potential to reduce unnecessary antibiotic use. Here we compare the performance of the new assay* against the laboratory-based assay** for the detection of respiratory viruses.

Method: A total of 109 archived clinical samples sent routinely to the Virology laboratory, and already tested on the laboratory-based assay**, were retrieved from -80°C freezers and tested on the new assay*. Sensitivity and specificity for the viral targets were calculated with the laboratory-based assay** as the gold standard.

Results: Compared to the laboratory-based assay**, the respective new assay* sensitivities/specificities were: 87.5%/100% for influenza A; 71.4%/100% for influenza B; 100%/96.2% for coronavirus; 83.3%/100% for adenovirus; 58.3%/100% for bocavirus; 75%/99.2% for human metapneumovirus; 71.4%/100% for parainfluenza (PIV)-1; 60%/100% for PIV-2; 75%/100% for PIV-3; 100%/99.25% for PIV-4; 84.6%/100% for RSV; 84.6%/97.6% for rhino/enterovirus. Overall, from this laboratory-based evaluation, the new assay* was slightly less sensitive on these archived frozen samples than the laboratory-based assay** for most viral targets, though this could have been due to the effects of the freeze-thaw cycle.

Conclusions: The molecular POCT*** shows relatively good sensitivity/specificity for most respiratory virus targets, which may be improved when tested on fresh samples. Currently, two ward-based evaluations are ongoing, where fresh samples will be tested and compared on these two assays, without a freeze-thaw cycle impacting on the new assay* results.

*Qiagen QIAstat-Dx POCT
**AusDiagnostics (16-well, cat. 20602)
***QiaStat
FIRST EVIDENCE OF NEUTRALIZING MERS-COV ANTIBODIES AMONG A HIGH-RISK POPULATION IN MOROCCO.

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6Who, Geneva, Switzerland
7The University of Hong Kong, Hong Kong, Hong Kong

Aim: “The Middle East Respiratory Syndrome of Coronavirus (MERS-CoV) remain a major concern for global public health, causing accurate respiratory illness (ARI). Dromedaries represent the major reservoir host of MERS-CoV and the source of human infection. The aim of our study is to assess the seroprevalence of MERS-CoV infection in presumed high-risk population of Morocco in close contact with dromedaries.”

Method: “This investigation was carried out in three study sites in the south of Morocco with high-density of dromedaries. A survey was adapted from WHO questionnaire for infection risk factors assessments. Three categories of participants were set; general population, slaughterhouse workers and herders group. Human sera samples were tested for anti MERS-CoV IgG antibodies through ELISA, pseudoparticule Neutalisation and plaque reduction neutralisation assay.”

Results: “479 blood sample were collected from November 2017 through January 2018. Our results highlight the first evidence of human primary cases of MERS-CoV infections with documented close direct exposure to dromedaries in Africa. The seroprevalence of MERS-CoV was low (0,82%), 3 of 137 slaughterhouse workers and 1 of 186 from general population group were MERS-CoV neutralizing antibody positive.”

Conclusions: “This is the first study focusing on MERS-CoV transmission risk factors in Morocco through a seroprevalence study among high-risk population and providing evidence of zoonotic transmission of MERS-CoV in Morocco.”

Acknowledgement: “This study was supported by funding from WHO-EMRO and a contract HHSN272201400006C from National Institute of Allergy and Infectious Disease, National Institutes of Health.”
[P034] HOW TO COPE WITH THE DRAMATIC INCREASE OF RESPIRATORY SAMPLES DURING THE WINTER SEASON?

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Aim: Winter seasons see a dramatic surge of respiratory samples (RS) in virology laboratories. The current challenge is to find the right compromise between efficacy and cost while providing a quick answer to clinicians. We report our experience using complementary automated processes during last winters.

Method: Between 2017 and 2019, our diagnostic strategy for respiratory infections evolved toward rapid molecular testing¹ coupled with multiplex one-step real-time RT-PCR based methods² either fully manually, then on two automated solutions³.

Results: Year-round, the number of RS handled per week varies from 1 to 331, corresponding to a median 4-fold increase during winter months. To limit the cost, the percentage of rapid testing over classic RT-PCR for flu/RSV was reduced from 38% to 30% between 2017 and 2019. Using a single run per day performed on primary tubes, the multiplex automated approach helped to release as many as 2625 results generated on heterogeneous RS types during the short epidemic period. Overall quality was improved, turn-around-time reduced without technical failure. Complementary panels covering up to 16 viruses, allowed to quickly adapt to the demand by using a single panel targeting Flu A/B (including subtyping) and RSV when necessary. Evolving from the fully manual to the latest automated process, abrogated all technical failures while decreasing time-to-results.

Conclusions: Rapid adaptation to the epidemiological flu season is mandatory. According to our experience, a fully automated solution supported by a rapid molecular method was the most efficient strategy.

¹GeneXpert (Cepheid); ²Seegene Allplex, Nimbus and Starlet Systems (Eurobio-Seegene)
**[P035] DETECTION AND SUB-TYPING OF ENTEROVIRUS D68 STRAINS IN RESPIRATORY SAMPLES COLLECTED IN FOUR UNIVERSITY HOSPITALS IN GERMANY, 2015-2018**

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**Aim:** “Enterovirus D68 (EV-D68) mainly causes respiratory disorders but has also been associated with acute flaccid myelitis (AFM). In 2014, an EV-D68 upsurge coincided with an increase of AFM cases in the USA. In 2016, a new upsurge of EV-D68 cases was detected in Europe and the USA with the emergence of a divergent B3 lineage as well as in 2018, when in addition to the B3 lineage also A2/D1 strains were reported from France and Italy.”

**Method:** “In the framework of the German RespVir network, we set up a laboratory based surveillance of EV-D68 by retrospective typing of enterovirus positive respiratory samples collected in four tertiary university hospitals in Germany.”

**Results:** “From 2015 through 2018, 51,391 respiratory samples were screened for enteroviruses, with 725 (1.4%) being positive. Using VP1 for molecular typing, 117 of these were identified as EV-D68: 7/116 (6%) in 2015, 89/238 (37.4%) in 2016, 6/174 (3.4%) in 2017 and 15/197 (7.6%) in 2018. Sub-typing showed co-circulation of lineages A1 (n=2), B1 (n=1), and B3 (n=2) in 2015 but predominance of lineage B3 in 2016 (n=80) and 2017 (n=6) as well as re-emergence/co-circulation of A2/D1 in 2018 (n=4). Sequence analysis of the A2/D1 strains showed high nucleotide similarity to strains reported from France and Italy in 2018. As observed in 2013/2014, all A2/D1 strains were collected from adult patients, whereas B3 strains were identified mainly in children.”

**Conclusions:** “Altered epitope sites leading to reduced immunity against these strains could be responsible for this phenomenon.”
Aim: Nosocomial influenza virus infection is known to cause significant morbidity and mortality among hospitalized patients. Additionally, the disease burden of other respiratory viruses, like parainfluenzavirus type 3 (PIV-3), is high and severe disease courses can occur in young children and immunocompromised patients. The impact of nosocomial infections, however, is rarely studied systematically in viruses other than influenza. The aim of the study was to determine the rate and clinical characteristics of nosocomial infections with influenza viruses A/H3N2, A/H1N1, B and PIV-3 in five consecutive seasons.

Method: A retrospective observational study was conducted at Leipzig University Hospital (UKL) on patients with laboratory-confirmed infection with either influenza virus or PIV-3 from 1st October 2012 to 30th September 2017.

Results: A total of 638 patients were tested positive for influenza virus and 309 patients for PIV-3. Nosocomial infection occurred in 13.9% (n = 89) of all influenza cases. Rates of nosocomial infection differed between influenza virus types and seasons with up to 17.4% for influenza A/H3N2 and up to 22.2% for season 2016/17, respectively. Regarding PIV-3, 23.9% (n = 74) of the cases were nosocomial and rates did not vary significantly between the seasons. For both, influenza virus and PIV-3, community acquired and nosocomial cases differed in underlying medical conditions and immunosuppression.

Conclusions: Nosocomial infections account for a substantial proportion of infections with influenza viruses and parainfluenzavirus type 3 cases. Knowledge of the baseline rate of nosocomial viral infections could contribute to the implementation of appropriate infection control measurements.
[P037] THE EFFECT OF IMPLEMENTATION OF RAPID MOLECULAR DIAGNOSTIC PANEL FOR RESPIRATORY PATHOGENS

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Aim: To assess the effect of implementing a rapid molecular test (RMT) for respiratory pathogens in a university hospital.

Method: A commercially available RMT for respiratory pathogens* was used as an alternative to the routine multiplex qPCR assay** between January and March 2019. The request for the rapid test was limited to the infectious disease specialists and emergency department physicians while there was no restriction for the routine test. RMT results were delivered to clinicians by a phone call while results of the routine test were reported through hospital information system.

Results: During 3-month period, 95 samples tested by RMT and 341 by the routine test. In both groups, pediatric samples were the majority (55% vs 78%). Positivity rate was 58% and 73%, respectively. Compared to the routine test, RMT significantly (p<0.01) decreased the median turnaround time (2 hours vs 46 hours). There were 11 influenza-A positive patients in the RMT group, 6 of them received antiviral therapy after the result, one was given empiric oseltamivir, while the therapy decision of the four outpatients were missing at the medical files.

Conclusions: It is expensive to use Respiratory Panel* in routine diagnostics in our country. However, maximum patient benefit has been achieved by using it for specific patients and delivering results to the clinician by phone which greatly reduced the turnaround time. The effect of RMT on the use of antimicrobial therapy has been evaluated partially, suggesting the need for a better monitoring system.

*DiagCORE, QIAGEN
** Respiratory Pathogens 21, FTD
Aim: Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis in infants. The impending introduction of RSV surveillance programmes, coupled with a number of RSV vaccine candidates and monoclonal antibody prophylactic treatments in various phases of clinical trials, means a deeper understanding of RSV is imperative.

Method: A proportion of RSV positive specimens, received for diagnostic testing (2015 – 2018) by the National Virus Reference Laboratory, Ireland, were selected for analyses (n=337). Genotyping was based on partial coding sequence of the glycoprotein (G) gene. Amino acid sequences were aligned with subunit vaccine epitope candidates and putative therapeutic antibody binding sites.

Results: RSV A and RSV B were shown to co-circulate each season. Phylogenetic analysis detected a high degree of variability between the circulating RSV strains, at the nucleotide and amino acid level. The ON1 insertion was detected in the majority of RSV A strains. All RSV B sequences clustered as the BA genotype. A comprehensive analysis of the amino acid residues showed that, despite the high-level variation seen, a number of putative therapeutic antibody epitopes and subunit vaccine candidates were highly conserved for both subtypes.

Conclusions: This study provides baseline genotypic surveillance data of RSV, which will facilitate the introduction of an RSV genotypic surveillance programme. And in addition, demonstrates that, despite the large amount of genetic diversity within circulating RSV strains, the binding sites for potential vaccine candidates and antibody therapies are highly conserved. Continued monitoring of RSV is essential to inform vaccine and treatment efficacy post introduction.
Aim: Inappropriate prescribing of antibiotics to treat misdiagnosed viral infections has likely added to the burden of antimicrobial resistance. The global incidence of respiratory tract infections is estimated to affect 3.9 million people annually. Therefore, improvements in respiratory viral infection diagnostics can assist in supporting antimicrobial stewardship programs. Clinical diagnosis has undergone a paradigm shift toward molecular assays. Point-Of-Care tests promise short turnaround times without the need for laboratory trained staff, expediting clinical decision making and improving patient management, however this can only be achieved if all assays are of similar sensitivity.

Method: The standardisation of Influenza and RSV molecular assays using internationally recognised reference preparations has the potential to harmonise the analytical sensitivities of different methods, allowing better inter-assay comparability through the setting of minimal detection limits.

Results: Studies report variable sensitivities of different POC tests, operating better with higher viral loads, risking misdiagnosis of lower viral load samples. Classical molecular assays have also shown large variabilities. Data gathered by NIBSC for Influenza A & B run controls demonstrated high inter-laboratory variability (CT values range 25.30 - 40.66 for Influenza A (H1N1), 24.30 - 37.88 for Influenza A (H3N2) and 22.46 - 36.75 for Influenza B). Furthermore, data reported in 2011 from an EQA panel highlighted differences in amplification efficacy of both Influenza and RSV between multiplex commercial assays.

Conclusions: NIBSC is identifying suitable strains of Influenza A/B and RSV A/B to evaluate in POC and traditional molecular assays to evaluate their suitability as WHO International standards.
Aim: “Every year, 30,000 to 40,000 people with flu-like symptoms have been registered in the Federation of Bosnia and Herzegovina (F&H). The study was designed to evaluate epidemiological and virological characteristics of the flu season during the 2017/18 in FB&H.”

Method: “Nasopharyngeal swabs were collected from patients with influenza symptoms and examined for influenza A and B viruses using a real-time RT-PCR.”

Results: “Influenza virus was confirmed in 184/386 (47.66 %) samples, with 67/184 (36.41%) of females and 117/184 (63.58%) of males. Influenza A virus was detected in 51.63% (95/184) cases with subtype pdm09 (H1) 88,42% (84/95) as dominant and only 11,57% (11/95) of influenza A (H3) subtype. Influenza B was detected in 48,36% (89/184) cases. Participation of patients with SARI (severe acute respiratory infection) or ILI (influenza like illness) with confirmed influenza was similar (52% 31/59 vs. 48.1% 28/59). Only 1/59 patient with a SARI /ILI was vaccinated, 49/59 did not receive the vaccine, 4/59 were unknown, and 5/59 did not have any data. The average age of all influenza positives was 34,4 years. The share of influenza positive in the most affected age groups was registered in school-age children 22,8% (5–14 years; 41/184) and in patient over 65 years 15,21% (28/184).”

Conclusions: “There is a need for continuous surveillance in order to predict seasonal trends and prepare for a timely response to influenza outbreak.”
Aim: The aim of this study was to describe the epidemiology of picornaviruses, rhinoviruses (HRV) and enteroviruses (EV) in particular, in hospitalized patients under 18 years old in the Valencia Region from 2014/15 to 2017/18 seasons.

Method: From a prospective, active-surveillance hospital-based study, we selected admitted patients <18 years old (y.o.) from 2014/15 to 2017/18 seasons (November to April, September to June in the 2017/2018 season) with ILI. Samples were tested by RT-PCR. Patient information was collected by interviewing legal tutors and/or by clinical records review.

Results: A total of 388/1519 (26%) HRV infections were detected from the total patients by age group, 194 (30%), 56 (22%), 84 (24%) and 54 (20%) HRV infections were found in <1 y.o., 1 y.o., 2-4 y.o. and 5-17 y.o., respectively. Among those <1 y.o., the highest HRV incidence rate was detected in children 1 month of age and this fact was consistent across seasons. The length of hospitalization (LoH) was 3(2-5) days for HRV and 3(2-3) for EV. Patients infected by any of both picornaviruses were mainly admitted due to bronchial disorders.

Conclusions: HRV infection burden is much higher in younger patients admitted to the hospital for ILI, as compared with EV infections. These younger patients had longer LoH. Hospital admissions caused by picornavirus infection of the respiratory tract were mainly linked to bronchial disorders, although a high variability was detected among age groups and seasons.
[P042] MINORITY VARIANTS DETECTED BY WHOLE-GENOME NEXT-GENERATION SEQUENCING (WGS) AND SEVERE H3N2 INFLUENZA DURING THE 2016-17 AND 2017-18 SEASONS.

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⁵Public Health Agency of Catalonia, Barcelona, Spain
⁶Microbiology Service, Center of Biomedical Diagnostics, Hospital Clinic i Provincial, Universitat de Barcelona, Barcelona, Spain

Aim: We aimed at integrating patient and influenza whole genome sequencing (WGS) data, obtained with a rapid next-generation sequencing (NGS) assay for genetic virus characterisation in the context of epidemiological surveillance of severe influenza and Hospital cases during two consecutive seasons.

Method: Nucleic acids were extracted from nasopharyngeal samples, and a single-tube RT-PCR protocol was followed using universal primers for all viral segment RNAs. 45/82 patients included corresponded to severe cases -ICU admission and/or exitus- (27/53 for 2016-17 and 18/29 for 2017-18). Labelled PCR libraries were pooled in one run of sequencer* with the 2x150 Mid-output kit. We used an in-house automated bioinformatic pipeline for demultiplexing, QC, sequence pairing, assembly and variant analysis for each sample and segment of the viral genome. Validation of minority variants was performed using the INSaFLU platform (Borges et al. 2018).

Results: We obtained a mean coverage of >4,000 reads/nt. Although we did not detect any clear phylogenetic clustering of viruses from severe cases, the frequency of minority variants (present at less than 50% in the viral population, and accounting for the eight segments) was a significantly associated with severe influenza (U-Mann-Whitney test), in particular for the 2016-17 season.

Conclusions: Influenza WGS by NGS with automated variant analysis is useful for epidemiological surveillance of severe influenza cases and will allow for an exhaustive characterisation of viral mutational patterns linked to severe ILI. The association of minority variants with severe influenza cases may vary between seasons, and future investigation is warranted.

* Illumina NextSeq
DETECTION AND QUANTIFICATION OF CMV IN MULTIPLE MATRICES

Fanny Gelas

Biomérieux, R&d Molecular Diagnostic, Grenoble, France

Aim: "CMV can cause severe disease in immunocompromised patients including transplant patients. Congenital CMV is also the main cause of neurological handicap from infectious origin in children. PCR kit* allows its detection and quantification in whole blood, plasma, amniotic fluid, urine, cerebrospinal fluid and Broncho Alveolar lavage. Additional tests were done in the objective to extend performances to saliva, as well as to confirm (using WHO) existing ones in the current strengthened regulatory context."

Method: "Limits of detection (LoD) were determined using the 1st WHO CMV on all claimed matrices (testing 20 replicates per 3 concentrations). Linearity and precision were also assessed. Extraction was done using instruments and amplification on system***."

Results: “A common LoD of 290 (2.5 log_{10} cp/mL) is claimed for plasma, amniotic fluid, saliva swab, urine and CSF, and of 350 (2.5 log_{10} cp/mL) for whole blood and BAL, confirming those matrices as more complex. The claimed dynamic range is 500 (2.7 log_{10}) to 7.9E+07 (7.9 log_{10}) cp/mL for all specimen types, which was confirmed in linearity and precision studies."

Conclusions: “PCR kit* is currently used in laboratories for CMV detection and quantification in various specimens. Analytical tests done on all specimen types contribute, along with complementary clinical studies, to confirm and extend the range of validated specimens, offering to laboratories one single assay for various clinical matrices."

* The CMV R-GENE® assay (ARGENE®, bioMérieux)
** easyMAG® or EMAG
*** Applied Biosystem ABI 7500 Fast/Fast Dx systems
[P044] EXTERNAL QUALITY ASSESSMENT (EQA) PILOT STUDY FOR MOLECULAR DIAGNOSTICS OF TORQUE TENO VIRUS (TTV)

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¹Quality Control for Molecular Diagnostics, Glasgow, United Kingdom
²University of Pisa, Pisa, Italy
³Qcmd, Glasgow, United Kingdom

Aim: TTV is the most abundant component of the human virome and has been classed as an orphan virus, able to establish chronic infections in an extremely large part of people without causing overt pathology. TTV viremia is increased in immunosuppressed hosts, and it has been suggested that the measurement of TTV load following treatment could be useful to gauge the efficacy of immunosuppression. EQA supports development of reliable molecular assays. Here we present the results of the first EQA study in that field.

Method: QCMD distributes panels containing different concentrations of TTV to registered laboratories annually, to allow assessment of sensitivity and specificity of their routinely used molecular assays.

Results: Eleven laboratories registered with 7 returning results. Comments for not returning included ‘assay under development’ which may not be surprising as this is a target relatively new to routine diagnostics. Responding participants applied assays which were equally split between commercial and in-house developed. Qualitative assessment of the results showed that all participants could detect TTV. Quantitative results were also submitted by 6 of the 7 participants and quantification across dilution series showing accuracy in quantification.

Conclusions: This pilot study demonstrated that while this is a new developing field, laboratories that have introduced testing have done so at a high standard. It’s important that proficiency schemes are available to assist laboratories in meeting regulatory requirements. Laboratories should be aware of the limitation of their assays and perform their own validation and verification in line with ISO 15189 and other requirements.
Aim: We present 2 cases of immunosuppressed individuals who received live shingles vaccine* with a literature review and outline of management.

Method: Clinical data were collected during management and literature review conducted via PubMed.

Results: A 71-year-old renal transplant patient on long-term immunosuppression presented the day after receiving live shingles vaccine* in the community. Although the patient had detectable varicella zoster virus (VZV) IgG, the clinical decision was made to treat with valaciclovir for one week due to the high titre (19,500 PFU) of live viruses in live shingles vaccine*. The patient remained asymptomatic and weekly blood VZV DNA remained undetectable during the one-month post-exposure. Letters were sent to over 1800 immunosuppressed renal patients and their GPs restating the risks associated with live vaccines. A second 71-year-old sarcoidosis patient on long term prednisolone developed a multi-dermatomal rash 3 weeks after receiving live shingles vaccine*—which was confirmed to have been caused by the vaccine strain—and was treated with oral aciclovir. Although uncommon, there are reports of disseminated VZV caused by the vaccine strain, including one death in an immunosuppressed individual1.

Conclusions: Clinicians and patients should be aware of the contraindications to live vaccine, and steps to take in the event of inadvertent administration. A glycoprotein E sub-unit shingles vaccine**, currently in phase 3 clinical trials, may be safer in the immunosuppressed.

References

*Zostavax
**Shingrix
Aim: This paper describes the clinical course of 2 renal transplant recipients who acquired hepatitis E (HEV) from the same donor.

Method: One week after transplantation the renal team was informed the donor had tested positive for HEV RNA (viral load of 3000 IU/ml). HEV IgM and IgG were negative, indicating early infection. Monitoring of recipients was commenced in line with national guidelines to identify HEV viraemia (PCR) and immune response (serology).

Results: Both recipients, who were HEV IgG negative pre-transplant, became viraemic, developing mildly raised ALT. Sequencing identified both as having HEV genotype 3, group 2. High homology between the strains supported a common source of infection. One patient developed low level HEV IgM low level, with no impact on viral load. The second patient developed IgM and IgG and the viral load dropped from $10^7$ to $10^5$ IU/ml but then stabilised. Due to persistent infection they were commenced on oral ribavirin at post-transplant day 136 (first patient) and day 170 (2nd patient). Both responded to ribavirin, with HEV RNA becoming undetectable in blood and stool. They are being followed up monthly to determine if they will achieve sustained virological response (i.e. HEV RNA negative 6 months after treatment).

Conclusions: This is the second cluster of HEV infections identified since the introduction of screening for HEV RNA in organ donors in 2017. The transmission of HEV and development of persistent infection in the recipients justifies the current screening guidelines and the recommendation to inform patients of the risk.
Aim: “We are reporting on case of severe drug-resistant HSV-1 systemic infection in 61-year-old male suffering from Darier’s disease. Skin lesions get infected with HSV-1 and progressed to second degree lesions over 20% of body surface area. Despite high dose aciclovir (ACV) therapy disease progressed to pneumonitis.”

Method: “Whole blood, bronchoalveolar lavage (BAL) and swabs from lesions were tested by real-time PCR*. HSV UL23 and UL30 genes were sequenced on ABI instrument, obtained data were analyzed with SeqScape Software v3.0 using HSV-1 17 as reference strain. The detected aminoacid substitutions (AAS) were compared with HSV Resistance database (Sauerbrei et al.). Swabs were cultivated using human embryonic lung fibroblast.”

Results: “The HSV-1 viral loads at the time of onset of pneumonitis (after 10 days of i.v. ACV therapy) were as follows: blood 1790 cp/ml, BAL 2 490 cp/ml and more than 10^7cp per reaction in swab. After 5 days of foscarnet therapy HSV was undetectable. 1 AAS of unknown impact in UL23, 9 AAS in UL23 and 6 in UL30 previously classified as polymorphism were identified. AAS V246M (UL 23) was detected in all 3 types of biological material.”

Conclusions: “Sequencing of UL23 and UL30 has reliable turnaround time, but the previously undescribed AAS are challenging to interpret. Switch in therapy was beneficial for patient and led to dramatic improvement in clinical status. We failed to confirm the phenotype by plaque reduction assay, which serves as golden standard in our laboratory, if possible.”

*Artus HSV-1/2 PCR Kit, Qiagen
[P048] THE VALUE OF TORQUE TENO VIRUS (TTV) AS A MARKER FOR THE DEGREE OF IMMUNOSUPPRESSION IN ADULT PATIENTS AFTER HEMATOPOETIC STEM CELL TRANSPLANTATION (HSCT)

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Aim: The aim of our study was to assess the monitoring of TTV viremia after hematopoetic stem cell transplantation (HSCT) as a predictive marker of immune-related clinical complications.

Method: In a retrospective study, 2072 whole blood samples of 124 adults’ patients (September 2015 until April 2018) were tested for viral loads of TTV DNA by real time PCR within 300 days after hematopoetic stem cell transplantation (HSCT). Clinical and laboratory data were collected and statistically analyzed.

Results: Patients with an underlying lymphatic disease have significantly higher TT viral loads compared to patients with an underlying myeloid disease (p < 0.05). Complete remission prior to HSCT correlated significantly with higher TT viral loads after HSCT (p < 0.05). Myeloablative conditioning regimens led to significantly higher TT viral loads than intensity reduced conditioning regimens (p < 0.05). A higher ATG dose was associated with a significantly higher TT viral load. Otherwise, clinically relevant events such as virus reactivations (CMV, EBV, ADV), acute graft versus host disease (GvHD), imminent relapse or death as clinical outcome were not reflected in any significant differences of TTVirus load. TT viral load after HSCT does not strongly correlate with T cell, T suppressor cell, T helper cell, NK and B cell count.

Conclusions: Virus reactivations, GVHD and clinical outcomes could not be predicted by monitoring the TTV viremia. Therefore, TTV seems not to be suitable as a marker for the degree of immunosuppression nor as a prognostic marker for clinically critical events after HSCT.
STABILITY OF NUCLEIC ACIDS IN AMNIOTIC FLUID, URINE AND SALIVA SWAB FOR MOLECULAR APPLICATIONS

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Aim: For molecular diagnostic tests, the stability of specimens shall be defined to ensure accurate qualitative and quantitative results. The stability of specimens was tested for detection of nucleic acids from viral or bacterial targets in 4 storage conditions (+25°C, +2/+8°C, -15/-31°C, ≤-60°C; including freezing cycles). The specimens tested were amniotic fluid, urine and saliva swabs.*

Method: Three selected targets (RNA & DNA viruses and bacteria sensitive to variation of environment) were co-spiked at low concentration (close to the limit of detection) in negative clinical specimens (n=20). Fresh specimens were used to keep integrity of potential inhibitors / nucleases known to be impacted by freezing. Analysis was based on comparison of detection and/or quantification between tested time points and initial test with qualitative (same status) or quantitative (+/- 0.5 log cp/ml or +/- 1.67 Ct) criteria.

Results: While stability of saliva swabs is demonstrated when stored at +2/+8°C or frozen, a quick degradation is observed at +25°C. The DNA and RNA viruses in amniotic fluid are stable in all tested conditions. The best storage conditions for urine are the frozen ones (-15/-31°C and ≤ -60°C). Indeed, a degradation of RNA virus is observed at +25°C and +2/+8°C.

Conclusions: Those results underline the difference of stability from one specimen to another. The RNA virus appears as most sensitive to the storage conditions.

*SIGMA-Virocult®
Aim: Haemorrhagic cystitis (HC) is a common complication occurring in allogeneic hematopoietic stem cells transplant (HSCT) recipients with a reported incidence of 10% to 70%. It is a significant cause of morbidity and therapy-related mortality. BK polyomavirus persists long-life in the urinary tract with asymptomatic urinary shedding in healthy individuals. In 5-20% immunocompromised persons after HSCT the BKV high rate replication is associated with HC. Numerous studies of reconstitution of the immune system after HSCT have established the principal role of T cells in the control of viral replication and reactivation. We determined the parameters of reconstitution of anti-BKV T cells after HSCT in patients with and without HC.

Method: In vitro expanded PBMCs BKV specific T cells were characterized by IC-FACS and ELISPOT IFN-gamma.

Results: To analyse immune reconstitution of BKV-VP1 and LTag specific T cell immunity after HSCT the functional activity, antigenic specificity and phenotype of T cells responsible for virological and clinical response in nine patients was monitored and compared. The efficiency of virus clearance correlated with reconstitution of CD4 T cells. Virus specific T cells of patients with CD4/CD8 >2 have low expression of inhibitory molecules TIGIT and PD1, whereas inhibitory molecules were abundant on virus specific T cells of patients with CD4/CD8 <2.

Conclusions: Magnitude of IFN-gamma T cell response to VP1 (CD4 T cells) and LTag (CD4 and CD8 T cells) of convalescent patient depends on viral DNA load and duration of BKV viruria after transplantation. Support of grant NV17-31593A-AZV, CR is acknowledged.
Aim: “To determine the phenotypic susceptibility of non-genital HSV-1 isolates from immunocompromised patients to ACV using the MTT method. The viruses were isolated from 2002 to 2018.”

Method: “Susceptibility to ACV was performed using the MTT method. ACV at final drug concentrations ranging from 0.064 - 101.12 µg/mL was added to Vero cells after HSV infection. The cultures were incubated for 7 days at 36°C, following which the medium was replaced with MTT. After incubation, the MTT solution was replaced with dimethyl sulfoxide. The colour that developed was measured at 570 nm and the IC₅₀ was calculated. IC₅₀ < 2 µg/mL is interpreted as susceptible and IC₅₀ ≥ 2 µg/mL is interpreted as resistant, as per CLSI.”

Results: “Three of 37 HSV-1 isolates were resistant to ACV and their IC₅₀ ranged from 14 - 26 µg/mL. Of these, one strain showed ACV-resistance conversion from another strain isolated 10 days earlier from the same patient. It is not known if the patient had been given ACV. With the other two ACV-resistant strains, one was on ACV prophylaxis and one has an unknown ACV treatment status. The IC₅₀ of ACV-sensitive isolates ranged from 0.1 – 1.3 µg/mL.”

Conclusions: “Of the HSV-1 isolates from immunocompromised patients, 8.1% were resistant to ACV. Prolonged exposure to ACV as prophylaxis could have caused resistance development in 1 case. It is important to monitor ACV resistance in immunocompromised patients with HSV, given the high prevalence of ACV resistance and its association with therapy failure in these patients.”
Aim: "In the UK, all blood and organ donors/recipients are screened for HTLV infections. Since the UK is a low prevalence country for HTLV infections, an assay with high sensitivity and specificity is required. Samples with repeat reactivity are sent to a reference lab for confirmatory serological and molecular testing. This leads to delays in the use of the blood/organs and can result in organ wastage. We aim to assess whether a signal/cut-off (S/CO) ratio higher than the manufacturer’s recommendation of 1.0 is a reliable measure of HTLV I/II infection."

Method: "We conducted a five year retrospective analysis of 11,766 samples tested on the Abbott Architect rHTLV I/II assay. Reactive samples (S/CO > 1) were referred for confirmatory serological and molecular detection (Western Blot and proviral DNA) at the national reference laboratory. Electronic, protected laboratory and hospital patient databases were employed to collate data."

Results: "Less than 1% (n=45/11,766) of samples were initially repeatedly reactive. Of these, 43% (n=19/45) had an S/CO ratio > 20, with infection confirmed in n=18/19 and indeterminate confirmatory results in n=1/19. No samples with an S/CO ratio < 4 (48%, n=22/45) or 4-20 (9%, n=4/45) had positive confirmatory results on subsequent testing."

Conclusions: "Samples with an S/CO >20 likely represent a true HTLV I/II infection. Reactive samples with an S/CO < 4 were unlikely to confirm for HTLV infections. Interpretation of these ratios can assist clinicians in the assessment of low reactive samples and reiterates the need for faster access to confirmatory testing."
Aim: BK virus allograft nephropathy is a major complication of kidney transplantation that markedly reduces graft survival. The aim was to investigate the presence and viral loads of BKV DNA retrospectively, to provide an overview of the clinical manifestations, diagnosis and related complications.

Method: The study included 30 KTRs who transplanted between August 2015 and May 2018 in Gazi University Faculty of Medicine. Demographic, clinical and laboratory characteristics of the patients were registered. BKV DNA was performed by Real-Time PCR in 135 specimens. Viral nucleic acids were isolated using Virus DNA Kit. Viral DNA was amplified with BK Virus Real Time-PCR Kit.

Results: Thirty kidney transplant recipients (KTRs), 53.3% (16/30) male and 46.7% (14/30) female, between 9-18 years were included in the study. Total BKV DNA positivity were found as 33.3% (10/30) in kidney transplant recipients. Of these patients, viruria was detected as 33% (10/30), 4% (13) of whom also developed viremia. In three of the BKV DNA positive patients, kidney rejection has occurred. Kidneys transplanted from 66.7% (20/30) of alive donors and 33.3% (10/30) of cadavers. All the BKV DNA positive patients were transplanted kidney from alive donors. BKV DNA viral loads of four BKV positive patients were 102 copy/ml, of three were 103 copy/ml, of two were 104 copy/ml and of one was 105 copy/ml. Cidofovir was used for the treatment of BKV DNA positive children.

Conclusions: Serial monitoring for BK virus replication is important for detection of BK infection. Detecting and clinical surveillance BKV DNA by Real-Time PCR for KTRs was important for the early diagnosis and treatment. Early BK detection appears crucial to prevent impairment of kidney function and subsequent graft loss.
[P055] GANCICLOVIR THERAPEUTIC DRUG MONITORING IN TRANSPLANT RECIPIENTS

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Aim: Insufficient (val)ganciclovir exposure can cause treatment failure, acquired drug resistance and side-effects. For adequate drug exposure, therapeutic drug monitoring (TDM) is an easy-to-use tool to guide dosing. The aim of this study was to gain more insight into the pharmacokinetics of (val)ganciclovir in routine care practice, in order to improve treatment and TDM practices.

Method: An observational study was performed in solid organ and stem cell transplant recipients receiving (val)ganciclovir as prophylaxis or treatment for CMV or HHV-6. Dosing was based on indication, body weight and adjusted for renal function, after measurement the pharmacist provided a recommendation. The predefined therapeutic window for ganciclovir C\text{trough} was 1-2 mg/L for prophylaxis and 2-4 mg/L for treatment, >5 mg/L was considered toxic.

Results: Sixty-six patients were enrolled resulting in 234 C\text{trough}. We obtained a median of 3 (IQR 2-5) samples per patient. The median C\text{trough} was 1.1 (IQR 0.4-2.2) mg/L for CMV prophylaxis and 2.3 (IQR 1.5-2.9) mg/L. For prophylaxis, 37 C\text{trough} (27%) were within the targeted range, compared to 54 C\text{trough} (55%) for treatment. Overall, five C\text{trough} were >5 mg/L, being either a first dose or after dosage adjustment. For 65 low C\text{trough} and 12 high C\text{trough} a dosage adjustment was advised. From the 77 recommendations, dosage was changed in 44 C\text{trough} (57%). Twenty-four follow-up C\text{trough} (55%) were adequate.

Conclusions: Despite a priori dose optimization an inter-individual variability of ganciclovir concentrations was observed. As only approximately 50% of the samples were in therapeutic range, conventional dosing could be aided by TDM.
Aim: In this prospective longitudinal study, relation between immunological reconstitution and infectious risk in hematopoietic stem cell transplant recipients (HSCTRs) was investigated.

Method: Twenty-three allogeneic HSCTRs were prospectively enrolled. Leukocytes, granulocytes, platelets and lymphocytes reconstitution, as well as the incidence of viral opportunistic infections (OIs), were monitored after transplant.

Results: CD4+ and CD8+ T-cell counts were low until 180 days post-engraftment, despite the complete reconstitution of leukocytes and platelets. Fifteen patients of 23 (65.2%) showed a CD4+ T-cell count at 30 days after engraftment <100 cells/µl while in the other 8 (34.8%) it was ≥100 cells/µl. In the first group a significantly higher number of patients developed at least one episode of OIs (12/15; 80% vs 2/8; 25%, p=0.0228). The occurrence of treated HCMV reactivations was higher in this group (10/15; 66.7% vs 2/8; 25%, p=0.0894). Fourteen patients of 23 (61%) showed a NK count at time of engraftment <70 cells/µl while in the other 9 (39%) it was ≥70 cells/µl. In the first group a significantly higher number of patients developed at least one episode of OIs (12/14; 85.7% vs 2/9; 22.2%, p=0.0066). The occurrence of treated HCMV reactivations was higher in this group (11/14; 78.6% vs 1/9; 11%, p=0.0028). HCMV-specific T-cell response against pp65 and IE1 remained low until 90 days post-engraftment. In this period, 69.6% of HCMV-seropositive HCTRs exhibit HCMV reactivation.

Conclusions: Evaluation of immunological response may represent a useful approach for early identification of HSCTRs with high risk of OIs, especially HCMV infection.
EVALUATION OF THE ANALYTICAL PERFORMANCE OF THE NEW HSV1 AND HSV2

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Aim: “The new HSV1&2 Real-Time PCR kit* provides a triplex reaction HSV1/HSV2/Internal Control to detect, quantify and differentiate HSV1 and HSV2 and a duplex reaction VZV/Internal Control. Performance (Limit of Detection-LoD and dynamic range) on CerebroSpinal Fluid (CSF), BronchoAlveolar Lavage (BAL), Whole Blood (WB), blood plasma and swab samples (mucocutaneous, anogenital and throat swabs) determined for HSV1 and HSV2 are presented.”

Method: “LoD were determined by serial dilution of ATCC strains into negative clinical specimens (20 replicates per concentration). Linearity (for quantitative claim) was tested from high positive clinical sample or culture, and precision assessed on 3 or 4 different concentrations for qualitative or quantitative claims respectively. All tests were done using the systems** and abi 7500 Fast/Fast Dx.”

Results: “The claimed LoD (95%) in cp/mL are:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>HSV1</th>
<th>HSV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB, blood plasma &amp; BAL</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>CSF</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Swabs (mucocutaneous, anogenital, throat)</td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

Combination of linearity, precision (as well as accuracy, not presented here) results allowed to determine the following range of quantification in cp/mL:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>HSV1</th>
<th>HSV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB, blood plasma &amp; BAL</td>
<td>500 to 1.0E+8</td>
<td>100 to 1.0E+8</td>
</tr>
<tr>
<td>CSF</td>
<td>250 to 1.0E+8</td>
<td>100 to 1.0E+8</td>
</tr>
</tbody>
</table>

The detection is only qualitative for swab samples.”

Conclusions: “The multiple sample types validated, including new specimen types as whole blood and blood plasma, enable laboratories to easily manage various HSV testing needs with one test. Simultaneous detection and discrimination of HSV1 and HSV2 will improve laboratory workflow reducing the reagent usage and labor time.
*Product under development”

*VZV R-GENE® - ARGENE® kit (bioMérieux)
** EMAG® or NUCLISENS® easyMAG®
Aim: The major aim is to study the relationship between BKPyV genotypes and VP1 external BC loop mutation and the risk of BKPyV reactivation, and the course and severity of nephropathy after kidney transplantation.

Method: In this ongoing prospective study, the plasma and urine samples from kidney transplant recipients and donors were screened for the presence and viral load by qPCR specific for all BKPyV subtypes. The VP1 epitope of BKPyV isolates from urine were amplified by PCR and genotyped by direct sequencing and analyzed phylogenetically. The infection of multiple BKPyV genotypes were identified with BKPyV genotype–specific qPCR assays for each of the 4 genotypes.

Results: BKPyV viremia (>10⁴) and viruria (>10⁷) was detected in 6.3% and 21% patients, respectively. According to phylogeny analysis all BKPyV urine isolates were either genotype Ib-2 (69%), IVc-2 (27%) or genotype Ib-1 (4%). The amino acid changes were identified in BC loop of VP1. The analysis using BKPyV genotype–specific qPCR assays revealed absence of multiple infections. No statistically significant difference in plasma viral load of BKPyV genotype I and genotype IV were detected.

Conclusions: The preliminary data have shown that the reactivation of the genotype Ib-2 is quite frequent in renal transplant patients and was also the only genotype detected in patients who developed BKPyV-associated nephropathy in the scope of 6 months follow up in our study. This work is supported by grant 17-29992A from Czech health research council, Ministry of Health of the Czech Republic
ELEVATED TORQUE TENO VIRUS IS ASSOCIATED WITH PREMATURE DEATH AFTER LUNG TRANSPLANTATION

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Aim: "Torque teno virus (TTV) is believed to be a marker of immunocompetence, and its use as biomarker in immunocompromised individuals is being extensively explored by groups worldwide. We prospectively studied the use of a single TTV measurement in an effort to predict patient survival and the risk of developing chronic lung allograft dysfunction (CLAD)."

Method: "We performed a prospective study on 137 lung transplant recipients transplanted between June 2001 and December 2010. All samples were collected a minimum of one year after transplantation. DNA was extracted using the eMAG Nucleic Acid Extraction System (bioMerieux, France) and qPCR was performed using the Argene R-Gene TTV quantification kit (bioMerieux, France) on an Applied Biosystems 7500 (Thermofisher, USA) according to the manufacturer’s instructions. Differences in mortality and CLAD between the groups were compared using Kaplan-Meier plots and log rank tests."

Results: "17 (12.5%) of our patients had undetectable TTV and were excluded from further analysis. The remaining 120 were divided into two groups based on serum TTV DNA load, the cut-off between the groups was a 4.36 Log₁₀ copies/mL. Our study had a median follow-up time of 6.2 [IQR 5.7-6.4] years. Patients with TTV under 4.36 Log₁₀ copies/mL lived on average 6.19 years from sampling compared to 5.55 years in patients with elevated TTV (P = 0.05). There is however no relationship between TTV and CLAD (P = 0.84)."

Conclusions: "A single measurement of TTV can be used to identify patients at risk of premature death."
Figure 1. All Cause Mortality

Strata: TTV_groups=High TTV  TTV_groups=Low TTV

P=0.05

Aim:
Aim: “This retrospective study has two main aims, firstly to describe the molecular diversity of enterovirus infections in solid-organ and bone marrow transplant patients, and secondly to compare genotypes of non-transplant patients, also admitted to the University hospital, in the Netherlands between 2014-2018.”

Method: “A total of 313 samples (from 269 patients) were selected that matched the desired parameters (all enterovirus positive samples with a typing result). Differences between transplant and non-transplant patients were investigated in terms of genetic characterization, sample type and viral load during an enterovirus infection. Phylogenetic trees were created to compare sequence variation and ANOVA was used to determine significant differences between transplant and non-transplant patients.”

Results: “Enterovirus D68 had the highest detection amongst both populations (transplant and non-transplant patients) with the greatest diversity of transplant types (solid-organ and bone marrow) (figure 1). However, some genotypes such as EV-C109 and CV-A22 had higher detection in the transplant group. Non-transplant patients had an overall greater diversity of sample types found per genotype, with CV-A6 exhibiting the highest sample diversity for both populations.

Figure 1: The number and type of transplants found for each genotype after an enterovirus infection (2014 to 2018).

Conclusions: “In immunocompromised individuals, particularly in transplant patients, knowledge on genotypic information is sparse. This study could prove important in the diagnostics of transplant patients during a suspected enterovirus infection, particularly in the development of specific genotype testing which could be incorporated into the diagnostic workflow.”
AN UNUSUAL CASE OF LONG-TERM HERPES SIMPLEX VIRUS DNAEMIA IN A 1-YEAR-OLD CHILD

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Primary infections with Herpes simplex virus 1 (HSV-1) in young children are often asymptomatic. Amongst the symptomatic courses, gingivostomatitis is the most common manifestation and may be accompanied by a short-term HSV DNAemia. Here, we report a case of primary HSV-1 infection followed by long-term HSV DNAemia.

A 9-month-old girl presented with gingivostomatitis, respiratory failure, massive hepatosplenomegaly, ascites, thrombocytopenia, and fever requiring intensive care. HSV-1 DNA was detected in whole EDTA blood samples (2.6E5 geq/mL), oral swabs, and feces. A disseminated HSV-1 infection was diagnosed. Intravenous aciclovir was given for 3 weeks followed by discharge with oral aciclovir.

Six days later, the girl was readmitted due to reduced condition, clinical signs of bronchitis, laryngitis, hepatomegaly, and enlarged lymph nodes. HSV-1 DNA was detectable in whole EDTA blood (4.2E4 geq/mL) and throat swab (8.6E7 geq/mL). Intravenous aciclovir therapy was re-started. Within 8 days symptoms disappeared. Despite complete recovery of the baby, HSV-1 DNA at concentrations between approx. 5E3 and ≤1E3 geq/mL was further detectable in whole EDTA blood for 8 months, but not in plasma. Viral DNA was also detectable in throat swabs and in a lymph node biopsy. Due to an alteration of STAT1 (loss of function), the child underwent stem cell transplantation. HSV-1 DNAemia stopped after induction of immunosuppression.

The site of HSV replication resulting in prolonged detection of viral DNA or DNA fragments in blood remains obscure. Whether oral HSV infection served as source needs to be discussed, as well as the consequences of DNAemia for antiviral medication.
[P064] RAPID CONTROL OF A PARAINFLUENZA VIRUS 3 OUTBREAK AFTER IMPLEMENTATION OF A NEW SAMPLING METHOD ON A HAEMATOLOGY WARD IN A TERTIARY HOSPITAL IN THE NETHERLANDS.

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Aim: We describe the first reported human parainfluenza 3 (HPIV-3) outbreak at an adult haematology-oncology ward and the effect of introduction of a low patient burden sampling method as screening method on symptomatic and asymptomatic patients.

Method: Clinical and diagnostic data were prospectively collected from July to September 2016. A HPIV-3 rt-PCR on oropharyngeal rinse samples was validated and allowed frequent screening with minimal patient burden. Screening of all patients admitted to the haematology-oncology ward was implemented three times weekly and new patients were screened before admittance to the ward. HN genes of 30 HPIV-3 positive samples were sequenced for genotyping.

Results: The HPIV-3 outbreak affected 53 patients. Validation of HPIV-3 PCR on oropharyngeal rinse samples demonstrated an up to tenfold higher sensitivity compared to pharyngeal swabs. Twenty patients were asymptomatic at the moment of first sampling of which 11 remained asymptomatic during the outbreak. The average duration of shedding was 14 days (range 1-58). Asymptomatic patients had a shorter period of shedding. Median PCR cycle threshold (Ct) of symptomatic patients was significantly lower (n=30, Ct 35.0) compared to asymptomatic patients (n=23 Ct 41.0). Genotyping showed that all but 3 isolates clustered together.

Conclusions: Implementation of oropharyngeal rinse sampling was highly sensitive. Symptomatic patients showed significantly lower loads and longer shedding compared to asymptomatic patients. Genotyping showed that 90% of HPIV-3 belonged to the same transmission chain. Implementation of this screening method resulted in rapid control of a HPIV-3 outbreak on a haematology-oncology ward.
Aim: We aimed to compare BKV DNA measurements that were performed with two different commercial Real-Time (RT) PCR assays.

Method: Totally, 112 clinical samples (51 plasma and 61 urine) that had been tested previously in Erciyes University Hospital Laboratory (LAB1) then after were examined in Marmara University Hospital Laboratory (LAB2). Nucleic acid extraction and RT PCR protocols were performed with automatized systems in LAB1 and LAB2*. Spearman’s test and Kappa test were performed for the statistical analyse.

Results: In both laboratories, 49 (80.3%) urine samples and 29 (56.9%) plasma samples were positive, 9 (14.7%) urine samples and 21(41.2%) plasma samples were negative. Three positive results in urine samples that were studied in LAB1 were negative in LAB2. Only one plasma sample that was detected negative in LAB1, was detected positive in LAB2. Inter-test agreement of qualitative results of two RT PCR methods for both urine and plasma samples was perfect (respectively; $\kappa=0.828$ and $\kappa=0.960$). The correlation between quantitative assays was statistically analyzed for urine and plasma samples (Spearman’s rank test, $\rho=0.800$, $p=0.200$; $\rho=0.136$, $p=0.616$). There was no significant difference between quantitative assays. Sixteen samples showed more than a 1 Log10 variability.

Conclusions: Inter-test agreement of qualitative results of all samples was found perfect but there was no correlation between quantitative results of urine and plasma samples in two RT PCR methods.

* respectively; Artus BK Virus RG PCR Kit, Qiasymphony extraction system and Rotor Gene Q RT PCR Cycler, Qiagen, Germany; and BKV Elite MGB® Kit, Elite in Genius, Elitech
[P066] NO ASSOCIATION BETWEEN PRETRANPLANT LYMPHOPENIA AND CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANT PATIENTS

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Aim: To determine if pretransplant lymphopenia or lymphocyte nadir could be used as a predictor for cytomegalovirus (CMV) infection in renal transplant patients.

Method: Renal transplant patients monitored for CMV infection was included in the study. Plasma from renal transplant patients were analysed for CMV-DNA by PCR. Viral loads in plasma > 1000 IU/mL was categorised as a CMV infection. Clinical data and lymphocytes count were collected from medical records. Pretransplant lymphocytes were counted in blood samples within 24 hours prior to transplantation. Lymprocyte nadir within the first week following transplantation was also registered. Lymphopenia was defined as 0.5 *10⁹/L at nadir and 1.0 *10⁹/L prior to transplantation. For statistical analyses chi-square test was used.

Results: 73 renal transplant patients were included in the analysis of lymphocyte nadir following the transplantation. Three (6.9%) out of 43 patients with lymphopenia at nadir had a CMV infection, and 2 (6.7%) patients out of 30 with no lymphopenia had a CMV infection (p = 0.959). 71 patients were included in the analysis of pretransplant lymphopenia. One (5%) out of 20 patients with lymphopenia prior to transplantation and 4 (7.8%) out of 51 (p=0.674) patients with no lymphopenia was diagnosed with a CMV infection after the transplantation.

Conclusions: In our cohort, lymphopenia prior to transplantation or nadir after the transplantation was not a predictor for CMV infection.
[P067] EPSTEIN BARR VIRUS IN BIOPSIES OF LYMPHATIC GANGLION IN PATIENTS WITH HODGKIN AND NON-HODGKIN LYMPHOMA

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Aim: Identify Epstein Barr Virus in tissue-embedded paraffin including T and B cell non-Hodgkin lymphoma as well as Hodgkin lymphoma.

Method: Series of cases, a retrospective study, Cervical lymph node tissue samples were obtained and embedded, the tissue collection was processed for viral DNA extraction performed per column and qPCR was used for the detection of Epstein Barr virus with TaqMan Probes”

Results: The cervical lymph node biopsies of 10 patients were analyzed, of which in 40% of the individuals the Epstein Barr virus was detected and diagnosed with Hodgkin’s lymphoma, in 30% of the cases the presence of the viruses and were diagnosed with non-Hodgkin’s lymphoma.”

Conclusions: The results show that 40 percent of patients with Hodgkin and non-Hodgkin’s lesions are associated with Epstein-Barr virus infection, which is consistent with other reports in Latin America, in addition was possible identify of EBV of samples in fixed tissue non fresh.
ROTAVIRUS INFECTION PROMOTES EMT THROUGH TRANSCRIPTIONAL SUPPRESSION OF MIRNA-29B

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**Aim:**” The study focused on transcriptional modulation of miR-29b during infection with Rotavirus (RV), an important agent of infantile gastroenteritis with double-stranded RNA genome.”

**Method:**” miRNA microarray was performed using RNA from mock- or RV-infected MA104 cells. miR-29b level was examined by qRT-PCR using TaqMan chemistry. Deletion mutants of miR-29b promoter was cloned and transfected either in Caco2 or 293T cells.”

**Results:**” We have identified the mechanism by which RV suppresses miR-29b in Caco2 cells and target pathways involved in cell invasion, migration, apoptosis resistance and proliferation. Our results suggested that RV infection transcriptionally downregulates miR-29b expression by directly modulating p53 through non-structural protein 1 (NSP1). In silico prediction suggests that Tripartite motif-containing protein 44 (TRIM44) and Glycogen synthase kinase 3 beta (GSK3β) are the direct targets of miR-29b. TRIM44 is a deubiquitinating enzyme, plays crucial role in a broad range of biological processes, including cell proliferation, differentiation, development, morphogenesis, and apoptosis. We have observed that TRIM44 expression is enhanced in RV infected cells, and overexpression of miR-29b inhibits TRIM44. We found that upregulation of TRIM44 activated the phosphorylation of AKT and GSK3β pathways. Furthermore, we found that RV mediated suppression of miR-29b could upregulate the level of p-mTOR at 12hpi. Subsequently, exogenous expression of miR-29b inhibited TRIM44 as well as GSK3β and their downstream molecules and limits viral replication.”

**Conclusions:**” Taken together, our results suggested that miR-29b is transcriptionally downregulated during RV infection, which in turn, enhanced TRIM44 and GSK3β signaling pathway and promotes viral replication.”
Aim: “The objective of our study was to elucidate whether apart from Rotavirus A (RVA) we could detect also other rotavirus species in human stool samples and waste water.”

Method: “We examined the set of 1500 stool samples of patients with mainly gastrointestinal disorders with the use of sensitive RT-qPCR methods*. The samples were collected during 2016-2018 in five microbiological diagnostic laboratories. Apart from that we examined eight samples of waste water from the intake of two big urban waste-water treatment plants with the use of next-generation sequencing (NGS).”

Results: “RT-qPCR assay was negative for RVB as well as RVC in all tested stool samples from patients. However, NGS of waste-water samples confirmed the presence of all rotavirus species which can infect humans (RVA, RVB, RVC). We were able to detect different RV genotypes including the ones typical for animal hosts. Waste-water samples were also positive for rotaviruses in RT-qPCR with the viral load ranging from 4.105 to 4.106 genome copies/l (RVA) and 6,3 – 37 genome copies/l (RVC) of raw intake water.”

Conclusions: “From the results of NGS of waste-water samples we may conclude that RVC and RVB are present in the Czech Republic. However, the epidemiologic relevance of those rotaviruses is probably very low or the viral loads in clinical samples is below the limit of detection. This study was supported by grant No. 16-29937A from the Ministry of Health of the Czech Republic.”

* Primerdesign™ Genesig® Kit for RVA, RVB and RVC detection was compared with in-house RT-qPCR assay
EMERGENCE OF RECOMBINANT NOROVIRUS GIIP16-GII.2 AND NOVEL GII.4 STRAINS IN PATIENTS WITH ACUTE GASTROENTERITIS IN SOUTHERN GREECE

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Aim: The study endeavored to illustrate current trends in molecular epidemiology of noroviruses in children in South Greece.

Method: The study involved 35 faecal specimens collected throughout a 19-month period (1/2016 – 7/2017) from different children aged 1 month - 14 years old, with symptoms of acute gastroenteritis. These 35 strains were tested positive for the presence of norovirus antigen, using an immunochromatographic screening assay. Molecular verification and genotyping were followed, based on gene sequences of VP1.

Results: GII.2 was the prevalent genotype (15/35, 42.3%), closely followed by GII.4 (40%). Other genotypes included GII.6 (6%), GI.1 (6%) and two strains were identified as GII.3 and GII.14, respectively. Additional molecular analysis demonstrated that a great proportion of GII.2 strains (8/15, 53.3%) were linked with the recombinant GII.P16-GII.2 variants that have started to emerge globally in outbreaks since 2016, while a sample (1/15, 6.6%) is related to GII.P2. Three GII.4 strains displayed recombinations in their genomes; two were associated with GII.Pe (14.3%), while only one was linked with GII.P21 (7.1%). Phylogenetic resolution showed that eight of the 14 GII.4 strains exhibited the "Sydney 2012" variant, whereas the remaining 6 GII.4 strains presented significant genetic divergence from this strain.

Conclusions: The findings revealed the great diversity of norovirus strains, add further epidemiological information about the increasing predominance of GII.2 noroviruses and highlight the necessity of continuing surveillance. GII.4 noroviruses were still detected in a large percentage; most importantly, the recombinant strains (three GII.4 and nine GII.2) might represent emerging variants that warrant further investigation.
IMPLICATIONS OF ROTAVIRUS VACCINATION FOR THE DETECTION OF ROTAVIRUS AT A LARGE DIAGNOSTIC LABORATORY

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Aim: “Rotavirus is a leading cause of paediatric gastroenteritis and is a notifiable disease in Ireland. As of 1st October 2016, all children are offered the live-attenuated vaccine*, with uptake by Q3 2018 at 89%. We report the impact of vaccine introduction on rotavirus detection at a large diagnostic laboratory and subsequent changes required to the rotavirus screening method.”

Method: “Rotavirus results from children aged 2-24 months tested at the UCD National Virus Reference Laboratory (NVRL) from January 2015 to December 2018 were analysed retrospectively. Vaccine status was unknown, so vaccine eligibility was deduced from the date of birth. Nucleic acids were extracted from faecal samples by system** and tested for rotavirus by real-time RT-PCR. From December 2017 the PCR was modified to allow differentiation of wild-type and vaccine-derived rotavirus.”

Results: “In pre-vaccine years (2015-16), 853/4784 (18%) samples had detectable wild-type rotavirus. In 2017, 156/519 (30%) of vaccine ineligible children had rotavirus, while 68/401 (17%) of vaccine eligible children had either wild-type or vaccine-derived rotavirus. In 2018 all children were vaccine eligible and 14/638 (2%) had wild-type and 47/638 (7%) had vaccine-derived rotavirus.”

Conclusions: “Following vaccine implementation, wild-type rotavirus in paediatric samples submitted to the NVRL for testing decreased from 18% to 2%, a relative decrease of 89%. As the vaccine may be excreted for several weeks following vaccination, albeit at lower titres than a natural infection, we found it necessary to modify our molecular detection method to differentiate wild-type and vaccine-derived rotavirus to provide clinically significant results.”

*Rotarix
**Roche MagNAPure96
Aim: The aim of this study was to investigate the prevalence and seasonal distribution of rotavirus and enteric adenovirus in patients with acute gastroenteritis.

Method: Results of 1511 patients admitted to Ankara Training and Research Hospital with gastroenteritis between October 2017-March 2019 were investigated retrospectively. A chromatographic immunoassay detecting both viruses simultaneously was used according to the manufacturer’s instructions.

Results: Of the 1511 patients, 664 (43.9%) were female and 847(56.05%) were male and 36(2.3%) were under 18 years old and 18 years and older respectively. Number of Turkish and refugee patients were 1357(89.9%) and 154(10.1%) respectively. Viral antigens were detected in 357/1511 (23.6%) samples, 297/1511 (19.6%) were positive for rotavirus and 60/1511 (3.9%) were positive for adenovirus. Of the Turkish patients, 256/1357 (18.9 %) were rotavirus and 53/1357 (3.9%) were adenovirus positive. Of the refugee patients, 41/154 (26.6%) were rotavirus and 7/154 (4.5 %) were adenovirus positive. The highest prevalence of rotavirus and adenovirus according to age groups was determined at 12-24 (33.3%) months of age and 5-18(18.5%) years of age respectively. The highest prevalence of rotavirus and adenovirus positivity was in spring (40.7%) and in autumn (38.3%) respectively.

Conclusion: Rotavirus is the most common cause of gastroenteritis during infancy and childhood. Also, enteric adenovirus is an important cause of gastroenteritis during this period. Since these viral infections may have serious complications, rapid diagnosis is important and detection of both viruses among various populations may be useful for epidemiological purposes.

*(Alltest;Hangzhou AllTest Biotech CO,China)
STRATEGY FOR MOLECULAR DIAGNOSIS OF CHILDREN’S GASTROENTERITIS IN MICROBIOLOGICAL DEPARTMENTS

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Aim: The aim of our study was to determine the better strategy for microbiological diagnosis of gastroenteritis using molecular multiplex bacteriological and virological assays.

Method: 322 stool samples or anorectal swabs were collected prospectively from 301 diarrheic children (124 girls) aged from 4 months to 16 years who attended the pediatric emergency unit of the Toulouse University Hospital (France) between April and November 2018. Samples were extracted on Magna Pure 96™ (Roche) with DNA and Viral NA MagNA Pure 96 Small Volume Kit® and amplified on CFX96™ (Bio-Rad) using Amplidiag Bacteria GE® (B) and Amplidiag Viral GE® (V) kit Mobidiag (Genewave, France). These tests allowed the detection of Salmonella, Campylobacter coli/jejuni, Shigella/EIEC (enteroinvasive E. coli), EAEC (enteroaggregative E. coli), EHEC (enterohemorrhagic E. coli), EPEC (enteropathogenic E. coli), ETEC (enterotoxigenic E. coli), Yersinia enterocolitica/pseudotuberculosis/pestis and astrovirus, rotavirus, sapovirus, norovirus I/II, adenovirus 40/41.

Results: 137 samples were tested with both B and V assays simultaneously: 76 (55.5%) were positive for at least one pathogen: 51 were B+/V-, 15 were B+/V+ and 10 were B-/V+. One hundred and thirty-nine samples were tested with B assay: 50 (36%) samples were positive. 107/139 samples (including 46 (43%) B positive) were tested with V assay. The rate of positivity improved from 43% to 61.7% i.e 20 (18.7%) new positive samples. The remaining samples (n=46) tested with V assay only were positive in five (10.9%) cases.

Conclusions: Our study showed that microbiological diagnosis of infants’ gastroenteritis requires the use of both bacterial and virological multiplex molecular assays.
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**Aim:** The PantherFusion\textsuperscript{®} Open Access\textsuperscript{™} (HOLOGIC) allows implementation of laboratory-developed tests (LDTs), with fully automated sample extraction, real-time PCR, and result interpretation. We describe the development of a multiplex LDT for norovirus and rotavirus from feces on PantherFusion\textsuperscript{®} as a first report of a multiplex LDT using this system functionality.

**Method:** The LDT was intensively optimized for primer and probe sequences, salt concentration, and amplification protocol. Reproducibility was assessed using defined sample material and controls. Performance of the multiplex LDT assay was compared to commercial multiplex assays in clinical samples using suspensions and swabs.

**Results:** We were able to establish an optimized protocol for the valid detection of viral stool pathogens. We observed reproducible results of PCR alone and extraction + PCR combined. The LDT had a sensitivity comparable to or greater than a commercial comparison assay, and showed excellent linearity. There was 100\% concordance in 160 clinical samples. Results from suspensions and direct swab stool samples preparation methods were highly concordant in the LDT.

**Conclusions:** The performance of the described LDT on the PantherFusion\textsuperscript{®} system was stable and reproducible. The LDT showed excellent agreement with results of commercial CE-IVD multiplex assays in respect to sensitivity and specificity. Additionally, the LDT makes use of the fully automated workflow of the platform, the option of continuous sample loading, as well as fast and continuous sample reporting. This improves high throughput testing not only in outbreak situation.
Aim: Human norovirus is a major cause of epidemic outbreaks of acute gastroenteritis globally, often in health care settings, hotels and schools. The aim of this study was to analyse the temporal distribution and variation of norovirus strains causing outbreaks during 13 years (2006-2018) in the Valencian Community (Spain).

Method: A total of 140 norovirus outbreaks were analysed and confirmed by RT-PCR. RNA was extracted from stool samples with Trizol reagent (Life Technologies) and analysed by ORF1 (polymerase gene) and/or ORF2 (capsid gene) RT-PCR. Genotyping was carried out by sequencing PCR amplicons, and genotypes were identified by BLAST and by using the norovirus genotyping tool (http://www.rivm.nl/mpf/norovirus/typingtool).

Results: Norovirus strains causing outbreaks along 13 years followed an epochal evolution, with the predominance of genotype GII.4 Den Haag_2006b variant in 2006, followed by GII.4 variants New Orleans 2009 and GII.4 Sydney 2012 the next years. In 2015, strains GII.17 arose for the first time, causing many outbreaks during 2016-17. In the last years, GI.2, GI.4, GII.2 and recombinant strains GII.4 P4-2009/GII.4-2012 were predominant. Different type of settings was affected, mainly hotels, elderly homes and restaurants, causing high burden of disease and economic losses.

Conclusions: The temporal distribution of norovirus genotypes is very dynamic and difficult to predict. Recombinant strains have been increasingly identified and contributed to epidemic outbreaks. Surveillance of norovirus outbreaks is a key factor to better control these infections.
[P079] ANALYTICAL PERFORMANCE OF THE BD MAX ENTERIC VIRAL PANEL IN THE DIAGNOSIS OF VIRAL ACUTE GASTROENTERITIS

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Aim: To compare the analytical performance of the BD MAX™ Enteric Viral Panel (Becton-Dickinson) and standard diagnostic procedures (ICG, ELISA, in-house RT-PCR) for the identification of viral agents of gastroenteritis.

Method: From July 2018 to April 2019 a total of 1,339 faecal samples from patients with acute gastroenteritis were analyzed by the BD MAX™ Enteric Viral Panel (RUO version). Results from the BD MAX™ EVP were compared to those obtained by routine analyses of 1,136 samples by EIA for rotavirus, adenovirus (ICG Rotavirus + Adenovirus, CerTest Biotec), astrovirus (ProSpecT™ Astrovirus, Oxoid) and norovirus (in-house RT-PCR) during similar period of time one year before (July 2017 to April 2018).

Results: By using the BD Max™ EVP a total of 407 (30.4%) samples were positive for enteric viruses: 95 (7.1%) for rotavirus, 46 (3.4%) for enteric adenovirus, 67 (5%) for astrovirus, 150 (11.2%) for norovirus, and 111 (8.2%) for sapovirus. By the standard diagnostic assays a total of 229 (20.7%) samples were positive for enteric viruses: 66 (5.2%) for rotavirus, 42 (3.2%) for adenovirus, 56 (6.2%) for astrovirus, and 65 (5.9%) for norovirus. Sapovirus was not investigated by conventional RT-PCR.

Conclusions: Diagnosis of gastroenteritis using the BD MAX™ Enteric Viral Panel (BD) allows the simultaneous detection of enteric viruses with less labour and higher sensitivity than conventional diagnostic methods for rotavirus and norovirus, without ruling out possible variations in the incidence of the different viruses. This approach allows a better understanding of the real prevalence of viral agents causing acute gastroenteritis.
[P080] EPIDEMIOLOGICAL ANALYSIS OF IMPORTED NOROVIRUS GASTROENTERITIS IN CHINA: 2018

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Aim: “Several entry-exit ports in China have been reported cases of norovirus gastroenteritis through importation. To strengthen control and prevention strategies with scientific evidence and better access the risk, the study aims to describe epidemiological characteristics of imported cases.”

Method: “The study analyzed the epidemic situation by using active surveillance data collected at entry-exit ports and standardized questionnaires distributed to arriving travelers. Data were entered in Microsoft Excel 2010 for further analysis.”

Results: “Between December 11th 2017 to April 3rd 2018, 262 imported cases of norovirus gastroenteritis were detected from 12 ports across the country (Figure 1), of these, 90.5% (237) were imported from Thailand. 87.8% (230) had developed symptoms of diarrhea. 184 diet reports were collected, of these, 95.1% (175) had eaten seafood. The predominant age-group affected is 15-54 years, which accounts for 70.7% of total cases (Median Age: 32 years, Range: 3-81 years). Travelers flying with the same aircraft are likely to report a mass norovirus infection.”

Conclusions: “The study characterized the epidemiological profile of imported cases of norovirus gastroenteritis at entry-exit ports. Cases were mainly imported from Chinese tourists returning from Thailand and majority of them had eaten seafood. Overall, the risk of importing norovirus into China remains high and the virus may continue to be spread in local areas. Pretravel education, boarder screening, early warning, active surveillance should be strengthened.”

Figure 1 Profile of Imported Cases of Norovirus Gastroenteritis
Aim: Acute infectious gastroenteritis is one of the most common causes of approach to emergency departments and especially important in children because it can be severe. The most common cause of acute infectious gastroenteritis is viruses. Many molecular methods are used in the rapid diagnosis of viral gastroenteritis. Multiplex PCR assays are the most widely used methods. Using these tests, the pathogen can be detected quickly, but what to do if multiple agents are detected is being investigated. The aim of this study was to investigate the clinical effects of multiple agents.

Method: Between January 2018 and February 2019, 501 samples from patients admitted to Ege University Medical Faculty Hospital were included in our study. In these samples, rotavirus, norovirus 1 and 2, adenovirus, sapovirus and astrovirus were investigated using enteric viral panel*

Results: Of the 501 samples, 241 were single, 74 were dual, and 3 were triple agents. In multiple agents, 45 of 77 were norovirus 2 with rotavirus and these were the most frequently detected multiple agents. Fourteen of 50 patients with multiple agents were hospitalized with acute viral gastroenteritis. Of the 97 patients with rotavirus, 32 (33%) were hospitalized and 9 (30%) of 30 patients with rotavirus and norovirus 2 were hospitalized.

Conclusions: The rates were close to each other so that we could not find relationship between multiple agents and hospitalization. In order to obtain more information, it is necessary to examine samples of patients with and without symptoms.

* BD MAX™ Enteric Viral Panel (BD Diagnostics, Baltimore, MD, USA)
EVALUATION OF A MODIFIED EN ISO 15216 METHOD FOR THE DETECTION OF HEPATITIS A VIRUS AND NOROVIRUS IN READY-TO-EAT SLICED APPLES.

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Aim: Fresh-cut fruits and vegetables (FFV) are products that have been minimally processed and are ready for consumption. They are important vehicles for transmission of viral gastroenteritis and hepatitis A illnesses. FFV can become contaminated as a result of the transfer of enteric viruses from faecally contaminated hands to the food or from using contaminated water for crop irrigation or washing. The aim of this study was to generate method parameters for the qualitative detection of norovirus GI, GII and hepatitis A virus in ready-to-eat (RTE) sliced apples.

Method: RTE sliced apple samples were standardized to an analytical portion size of 25g and inoculated with norovirus GI, GII or hepatitis A virus. After incubation at 4°C, virus was extracted using the EN ISO method 15216-1:2017 with minor modifications. Viral RNA was detected by real-time RT-PCR with TaqMan probe.

Results: RTE sliced apple eluates caused a blockage in the nucleic extraction robot in 66.1% of the samples. Blockage was cleared by treating samples with a chloroform/butanol mixture. The method was found to be specific for the three viruses, with no false negatives or false positives. The limit of detection was evaluated at 750, 2500 and >10,000 genomic copies for norovirus GI, GII and hepatitis A virus, respectively. Extraction efficiencies up to 19.1% were obtained.

Conclusions: RTE sliced apples have a distinctive characteristic and require a further extraction step. This study highlights the importance of evaluating the performance characteristics of validated methods in different food matrices to extend their scope of application.
Aim: The outer capsid of rotaviruses is composed of the VP7 glycoprotein (G genotype) and the protease sensitive VP4 protein (P genotype) that forms the viral spikes. VP4 is processed by proteolytic cleavage into two subunits, VP5* and VP8*. VP8* is mainly involved in the attachment process. The aim of this work was to determine the prevalence and titer of serum antibodies against several VP8* genotypes in children and adults in Valencia, Spain.

Method: To achieve our aim the VP8* protein from the P [4], P [6], P [8], P [9], P [11], P [14] and P [25] genotypes were produced in E. coli as GST fusion proteins. These proteins were tested against 89 serum samples from children (n=41, aged 3.5 years in average) and from adults (n=48, aged 58 years in average) by ELISA.

Results: Ninety-six per cent of the samples were positive for at least one of the VP8* antigens. Except for P [11], no differences in the seroprevalence were found between children and adults. We found significant differences between adults and children in the antibody titers against the P [4] (p = 0.0140), P[8] (p = 0.0024) and P[11] (p<0.0001) VP8* genotypes, having the children higher antibody titers than adults. Interestingly, positive samples against rare genotypes such as P [11] (only in children), P[14] and P[25] were found.

Conclusions: Our results demonstrate the circulation of several rotavirus genotypes in the population of Valencia and that humans have specific anti-rotavirus serum antibodies also during adulthood, suggesting the occurrence of successive rotavirus infections in the course of life.
[P084] DETECTION OF COSAVIRUS IN A PATIENT WITH ACUTE FLACCID PARALYSIS AND IN SEWAGE SAMPLES IN GERMANY

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Aim: “Cosaviruses (CoSV) belong to the family Picornaviridae and five species (A, B, D, E, F) have been classified to date. Cosaviruses were first in stool samples collected from both, non-polio acute flaccid paralysis cases and their healthy contacts in Pakistan in 2008. Further studies showed detection in symptomatic and asymptomatic individuals. The etiological role of cosaviruses still remains unclear. Testing of sewage samples proved continuous circulation in several parts of the world, but little is known on cosavirus epidemiology since routine screening is not performed and seroprevalence studies are limited due to lack of virus isolates necessary for virus neutralization assays.”

Method: “Using NGS, we identified a HCoSV in a stool sample of a three-year-old child with travel history to Pakistan hospitalized with non-polio acute flaccid paralysis.”

Results: “We sequenced and analyzed the complete genome sequence and confirmed the presence of a novel HCoSV species D strain in the patient. No other pathogens were detected by NGS or conventional methods (Cell culture, PCR, serology). To evaluate the presence and diversity of cosaviruses in Germany, we screened 46 sewage samples collected at one sewage plant in Berlin between 2013 and 2018. 54.3% of the samples were tested positive for Cosavirus RNA and partially sequenced using NGS. We detected co-circulation of CoSV-A (n=27), CoSV –B (n=2), and CoSV–D (n=9), with up to three different CoSV strains in one sewage sample.”

Conclusions: “Further investigations and the use of NGS techniques will allow comprehensive epidemiological and phylogenetic studies of cosaviruses.”
A SUBTYPE OF CYTOMEGALOVIRUS US28 GENE IN COLORECTAL CANCER LESION IS ASSOCIATED WITH A WORSE OUTCOME

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Aim: “Human cytomegalovirus (HCMV) infection is present in colorectal cancer (CRC) cells. This study aims to investigate HCMV US28 genetic polymorphism in CRC and its association with outcome.”

Method: “A total of 200 specimens were collected from the Biobank of the Division of Colorectal Surgery, Taipei Veterans General Hospital. A primer pair, covering the whole US28 gene (seq. 1-1062), was redesigned. Combining polymerase chain reaction (PCR) and sequencing, the US28 gene in CRC samples was determined. Mixed US28 genes in one sample were resolved by cloning and re-sequencing. Phylogenetic analysis was conducted, and Neighbor-joining tree was used to reconstruct a tree with 1000 bootstrap. The clinical, pathological and survival data were analyzed for different US28 genotypes. We used the Kaplan-Meier method to estimate patients’ survival. Significant differences in the survival curves were evaluated with a Mantel-Cox log-rank test.”

Results: “HCMV US28 genes were detected in 80 of 200 tumor specimens (40 %). Four major genotypes (alpha to delta) were identified. There were 69 single genotypes and 11 mixed-types. For all the samples with sequencing data, there were 38 alpha, 7 beta, 6 gamma, and 16 deltas (2 samples not in the major group). Amongst the stage I, II, and III CRC patients (n=160) those who contained US28 gamma type revealed worse disease-free survival (DFS) than others (p=0.0158).”

Conclusions: “HCMV US28 gene can be classified into four major genotypes in CRC specimens. The presence of US28 gamma genotype was associated with a shorter DFS for the CRC patients.”
P086 PATHOGENESIS OF CERVICAL CANCER ASSOCIATED WITH HPV 16 AND 18

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Aim: High-risk human papillomavirus (HPV) genotypes 16 and 18 are associated with 80% of cervical cancer, the second most common cancer among women worldwide. The most important part in its carcinogenesis is attributed to HPV oncoproteins E6 and E7, which affect the cell cycle proteins. In this study we investigate the effects of E7 binding to host cell cycle proteins, B-myb and p130 in HaCaT cells.

Method: Co-immunoprecipitation, Western blot analysis, immunofluorescence microscopy, flow cytometry and quantitative-PCR were employed.

Results: Results showed that disruption of the p130 expression occurs through the binding of LXCXE motif of HPV16 and 18 E7. This subsequently causes G0/G1 transcriptional activation as verified by cell cycle analysis. Concurrently, B-myb expression in HaCaT-HPV16 and 18 E7 was notably abundant due to the irregularities of host cell function. Immunofluorescence analysis exhibited cytoplasmic localization of p130 in the of HaCaT-HPV 16 and 18 E7 rather than nuclear localization as seen in normal cells prior to its proteasomal degradation. In addition, we also observed reductions in two differentiation markers, K10 and involucrin in HaCaT-HPV16 and 18 E7 cells.

Conclusions: These findings demonstrate that HPV infection allows continuous cell cycling with delayed cellular differentiation, one of the hallmarks of cancer. This excessive proteasomal degradation of p130 allows HPV to hijack the host’s cell cycle mechanism for its own replication and transforms them into oncogenic cells that ultimately results in cervical cancer.

Acknowledgement: This project is funded by the Ministry of Higher Education, Malaysia (FRGS FP015-2016)
THE ORGANOVIR NETWORK: BUILDING 3D CELL CULTURE MODELS FOR VIROLOGY

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Aim: Virus research currently relies heavily on 2D cell culture and animal models, which have limited potential for studying human-pathogen interactions. Recent advances in human 3D tissue culturing have created the opportunity to study virus-host interactions in a human setting. Organovir is a H2020 Marie Curie Trainingsnetwork of 15 partners, aiming to transform the virology landscape and establish human 3D cultures as superior models for viral pathogenesis studies and antiviral testing, thus replacing animal models and reducing animal use.

Method: Epithelial cells of the respiratory tract (RT) and gastro-intestinal tract (GIT) are important entry sites for pathogenic viruses. Human airway epithelium (HAE) cultures and lung organoids enable research of viral infection in the RT, while gut organoids facilitate studies on GIT viruses. HAE and organoids allow further studies on viral entry into the human body, which is an important point of intervention. Infections in secondary organs can be mimicked in 3D models of skin, lung and brain.

Results: We have established infections with several viruses on 3D cell models, and data from these studies correlate well with data in patients, showing the potential of 3D cell cultures as human models for infectious diseases. To improve 3D cultures for pathogen-host interaction studies, we developed a new model with fetal gut mono-layers on transwells.

Conclusion: In Organovir, advanced infectious disease models will be further developed by coculturing and coupling of tissues.

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Authors on behalf of the Organovir network (participants listed at www.organovir.com)
[P088] INHIBITORY EFFECTS OF ANDROGRAPHOLIDE ON EPSTEIN-BARR VIRUS REACTIVATION

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Aim: "Reactivation of Epstein-Barr virus (EBV) from latency into lytic phase allows the virus to spread among cells and has been reported to play an important role in development of several malignancies. Inhibition of EBV reactivation is considered to be a great benefit in treatment of virus-associated diseases. Andrographolide, a bioactive compound product purified from Andrographis paniculata has been reported to have inhibitory effect on herpesviruses infection as well as anti-tumorigenesis. This study aimed to examine inhibitory effects of Andrographolide on EBV reactivation in lymphoid and epithelial cell lines.”

Method: “Cytotoxic effect of Andrographolide on cells with EBV latency (P3HR1, AGS and HONE1) were analyzed by MTT assay. These Andrographolide-treated cells were induced to lytic phase by incubating with sodium butyrate for 24-48 hours. The mRNA levels of EBV immediately-early genes (BZLF-1 and BRLF-1) and early genes (BMRF-1 and BALF-5) were analyzed by quantitative real-time PCR (qRT-PCR). The ZEBRA protein of EBV was analyzed by western blot and the EBV production was analyzed by qRT-PCR.”

Results: “The EBV lytic phase was successfully induced within 48 hours as well as Andrographolide significantly inhibited the expression of EBV lytic genes in all cell lines. The expression of ZEBRA protein was significantly reduced in all cell lines in a dose-dependent manner. In addition, Andrographolide significantly reduced EBV production in all cell lines.”

Conclusions: “This result showed that Andrographolide might be a promising candidate for anti-EBV reactivation. The mechanism of inhibitory action is needed to further evaluate.”
EPSTEIN-BARR VIRUS PROMOTES STEMNESS OF ORAL SQUAMOUS CELL CARCINOMA

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Aim: “Epstein-Barr virus (EBV) is an oncogenic human herpesvirus that associated with various cancers. Recently, the prevalence of EBV-associated oral squamous cell carcinoma (OSCC) increases with an increased risk of OSCC. However, the role of EBV on the progression of OSCC is still unknown. Therefore, this study aimed to examine whether EBV is indeed to promote OSCC progression via an induction of OSCC stemness.”

Method: “EBV-positive tongue squamous cell carcinoma (SCC25 GFP/EBV) cells were previously established by cell-to-cell infection. This cell line was investigated for progression of OSCC tumor by characterization of stemness cell properties both in vitro and in vivo.”

Results: “Herein, EBV could promote mesenchymal properties of SCC25 GFP/EBV cells by inducing epithelial–mesenchymal transition (EMT), migration and invasion. Furthermore, EBV also induced expression of cancer stem cell markers, CD44 and CD133. The up-regulation of cancer stem cell markers was well correlated with tumor formation and growth in xenograft model. In addition, EBV further promoted capability of recapitulate tumor growth of SCC25 GFP/EBV cells, however, SCC25 cells (EBV-negative cells) failed to form tumor in xenograft models. Interestingly, a serial passage of tumor cells in mice increased malignant phenotypes of the tumor cells.”

Conclusions: “These results may provide a novel information of oncogenic roles of EBV in OSCC progression by induction of cancer stem cells.”
Aim: Human BK polyomavirus (BKPyV) is implicated in polyomavirus-associated nephropathy (PyVAN), a major cause of graft rejection in kidney transplant recipients (KTRs). Within the BKPyV genome mutations occur in the transcriptional control region (TCR). Our aim was to study their association with the development of PyVAN.

Method: We amplified complete BKPyV TCR from urine and plasma samples from fifteen KTRs with high BKPyV loads in blood and subjected them to massive parallel sequencing. The patients had either biopsy-confirmed PyVAN or presumptive PyVAN, or had no clinical evidence of PyVAN. Sequence rearrangements affecting cellular transcription factor binding sites, possibly affecting the efficiency of BKPyV replication, were specifically studied.

Results: The majority of sequences in urine as well as in plasma samples represented archetype BKPyV TCR. Occasional viral sequences harboring rearranged TCRs were detected in all three patient groups. However, substantially higher amounts of rearranged TCRs were detected in the urine and plasma of patients with biopsy-confirmed PyVAN. Specifically, the viral strains of one patient with biopsy-confirmed PyVAN strongly differed from other patients, as strains harboring rearranged TCR were found predominant.

Conclusions: Although archetype BKPyV predominated in most patients, higher proportions of rearranged strains were found in PyVAN patients as compared to non-PyVAN individuals. We conclude that archetype BKPyV can efficiently replicate in kidney epithelial cells, and TCR mutations seem not necessary for the development of PyVAN. However, immunosuppression may allow increased viral replication enabling the emergence of TCR variants with consequently enhanced replication efficiency.
[P091] MDM2 REGULATION OF P53 IN EPSTEIN-BARR VIRUS ASSOCIATED GASTRIC CANCER (PRELIMINARY STUDY)

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Aim: EBVaGC corresponds to 10% of all GC and is characterized by a distinct viral latency profile. EBVaGC has some distinctive features in terms of genome alterations, among them no p53 mutations. In a recent study from our group, we have shown that EBVaGC present a lower level of TP53 mRNA and higher level of p53 protein when compared with non-EBV associated cancers. This pattern of p53 expression is not well understood and some authors refer that the Murine Double Minute 2 (MDM2) protein, p53 major negative regulator, may be the explanation due to by some anomaly in MDM2 protein, leading to lack of degradation p53. The aim of the thesis study if to clarify the activity of MDM2 in p53 deregulation in EBVaGC.

Method: In this study we will use tumor tissues samples collected from IPO-Porto patients with EBV-positive and EBV-negative gastric cancers. DNA and RNA were extracted from tumor tissues collected from formalin-fixed paraffin-embedded (FFPE) tissue blocks and samples will be used for immunohistochemistry (ISH).

Results: Currently we are analyzing the MDM2 protein expression levels by two-step real-time PCR; performing the genotyping of MDM2 SNP309 polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); and protein accumulation in the tissue by IHC using specific monoclonal antibody.

Conclusions: This preliminary study intends to show if there is an association of MDM2 with p53 deregulation in EBVaGC.
[P092] OROPHARYNGEAL CARCINOMA ASSOCIATED WITH HUMAN PAPILLOMA VIRUS. CASE REPORT

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Aim: “To identify and genotype HPV in a Mexican woman with oropharyngeal carcinoma.”

Method: “A clinical inspection of the patient was performed, and supra-vital staining was performed with blue toluidine and was scheduled for an incisional biopsy, under local anesthesia with a 5mm punch. Tissue viral DNA was subjected to PCR analysis using the primers of MY09/11, GP5/6 and L1C1/C2 and the identification of genotypes and viral load was performed by multiplex PCR in real time (Seegene).”

Results: “Here we described a 55-year-old woman with sore throat since the placement of a dental prosthesis in the maxilla three months ago. A pathological history of diabetes mellitus II, hypertension, stenosis in the neural canal and smoker. The patient showed an erythematous plaque with whitish areas with irregular surface showing grooves, ulceration and finely papillomatous areas, which has diffuse edges soft consistency on the soft palate and the oropharynx on the right side; presenting mild pain on palpation. The histopathological study showed a malignant neoplastic epithelium detaching nests that infiltrate the lamina propria and push the fascicles of striated muscle, some nests had comedo-type necrosis and were accompanied by a moderate chronic inflammatory infiltrate. The molecular analysis gave results positive for HPV-6 with a “+” of viral load and diagnosed as conventional oral squamous cell carcinoma.”

Conclusions: “HPV-associated disease may not necessarily be transmitted via sexual practice patterns alone. This study reports the presence of HPV-6 and despite being of low risk, malignant progression to oral cancer is observed.”
Aim: Parvovirus B19 infection in pregnancy is mostly asymptomatic, but in about 3% of pregnant women can cause some fetal complications. Since pregnancy screening is not performed in Italy, seroprevalence data in our country are limited. We have therefore retrospectively assessed the seroprevalence of Parvovirus B19 in pregnant women in an urban area of northern Italy.

Method: From February 2017 to January 2018 we analyzed 1893 sequential sera from as many women: 1402 (74.1%) were Italian, 491 (25.9%) of foreign origin. Anti-Parvovirus B19 IgM and IgG antibodies were searched by LIAISON® chemiluminescence immunonoassays (DiaSorin, Italy) and the positive IgM samples were analyzed with an immunoblot (Mikrogen, Germany) and the Parvovirus-B19 genome (ParvoB19-DNA) was researched with molecular biology test (Elitech, Italy).

Results: Of the 1893 women, 1315 (69.5%) were positive for anti-Parvovirus IgG, 20 (1.1%) for IgM and 578 (30.5%) non-immune. Positive IgM women were also IgG positive. The difference in IgG seroprevalence between Italian (71.1%) and foreign (64.6%) is statistically significant (p <0.5). Of the 20 samples IgM positive at screening, 14 were confirmed positive at the immunoblot (of which 10 ParvoB19-DNA positive), one indeterminate (ParvoB19-DNA positive) and 5 negative (all ParvoB19-DNA negative), so the prevalence of positive or indeterminate IgM at immunoblot was 0.8%.

Conclusions: The seroprevalence of IgG anti-Parvovirus among pregnant women in our area is high and the presence of confirmed IgM indicates a possible risk of vertical transmission for which a clinical follow-up and further studies are indicated to highlight possible fetal consequences.
[P094] CYTOMEGALOVIRUS SEROPREVALENCE IN A POPULATION-BASED DIFFERENT SEX AND AGE GROUPS STUDY IN TURKESTAN REGION, KAZAKHSTAN.

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Background: Infection with CMV remains asymptomatic in most immunocompetent hosts and it is the leading cause of congenital viral infection worldwide. There is not any available study defining the number of individuals affected by CMV in Turkestan region in Kazakhstan. The aim of this study is to identify the seroprevalence of CMV from children and adult population in this region.

Material and Methods: Our study included population-based sample of 386 people, 192(49.7%) female and 194(50.3%) male, aged between 5 and 75. All blood samples were collected at Akhmet Yassawi University(Kazakhstan) and analyzed at Gazi University(Turkey). Serum samples were tested of CMV IgM and IgG antibodies using microparticulate chemiluminescent immunoassay(CMIA). The IgM/IgG positive samples were further tested for IgG avidity. CMV DNA were analyzed by Real-Time PCR.

Results: The overall seroprevalence rates for CMV IgG and IgM antibodies were 379(98.45%) and 6(1.55%), respectively. The IgG seroprevalence showed in significant differences between age groups: 39(92.8%) samples were from in the age group of 5–12 years; further, 42(93.3%), 114(99.1%), 148(100%) and 36(100%) were from age groups, 13–18, 19–40, 41–65 and 66–75 years, respectively. Using IgG avidity, CMV IgM seropositive samples was confirmed by a high avidity index(74%-98.8%). PCR results were also negative. Thus, primary infections were not detected.

Conclusion: The seroprevalence of CMV in this region is high compared with developed countries. All people in our country were positives with CMV until the age of 40. The factors that are largely related to the CMV seropositivity are the collective life of society and the traditional contact with kisses. Prevention of CMV infection in the population through hygiene counseling may lead to a decrease in CMV infections in this region.
[P095] QUANTITATIVE ANALYSIS OF PARVOVIRUS B19 BY REAL-TIME PCR METHOD IN PATIENTS WITH RASH

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Aim: In this study, it was aimed to investigate the presence of Parvovirus B19 DNA by real-time PCR in patients with rash and to evaluate the positive results according to demographic, clinical and laboratory findings.

Method: Thirty one patients with rash who applied to Ankara Training and Research Hospital between October 2018-March 2019 and whose Parvovirus B19 DNA quantitative tests were sent to Molecular Microbiology Laboratory were included in the study. Nucleic acid isolation from plasma samples was performed with Magnesia 16* and the target DNA was quantitated by montania 4896* with **Parvovirus B19 Quantification Kit v1* and the results were evaluated quantitatively.

Results: Parvovirus B19 DNA was detected in four/31 (12.9%) patients. Parvovirus B19 DNA quantitative values; demographic, clinical and laboratory findings are shown in the Table. All positive patients were from pediatric allergy outpatient clinic.

<table>
<thead>
<tr>
<th>Quantitative result (IU/ml)</th>
<th>Age</th>
<th>Sex</th>
<th>Other symptom</th>
<th>Lab (leukocyte count and CRP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.820</td>
<td>4 years 11 months</td>
<td>Male</td>
<td>Pruritus</td>
<td>8,19 x 10^9/L - 1,1 mg/L</td>
</tr>
<tr>
<td>6.671</td>
<td>8 years 2 months</td>
<td>Female</td>
<td>Pruritus</td>
<td>10,39 x 10^9/L - 23,7 mg/L</td>
</tr>
<tr>
<td>19.910</td>
<td>4 years 5 months</td>
<td>Female</td>
<td>Cough Erythema</td>
<td>6,09 x 10^9/L</td>
</tr>
<tr>
<td>56.990</td>
<td>12 years 5 months</td>
<td>Female</td>
<td>Erythema</td>
<td>6,12 x 10^9/L</td>
</tr>
</tbody>
</table>

Conclusions: For parvovirus B19 diagnosis and follow up especially in children with typical rash, B19 DNA based methods have become more common lately besides the screening of specific IgG and IgM antibodies.

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DETERMINATION OF SEROPREVALENCE ANTIBODIES FOR RUBELLA AMONG THE POPULATION OF TURKESTAN IN KAZAKHSTAN.

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Aim: “There is not any available population based study defining the number of individuals affected by Rubella in Turkestan region in Kazakhstan. The aim of this study was determination of seroprevalence antibodies for Rubella in this region.”

Method: “Our study included population-based sample of 386 people, 192(49.74%) female and 194(50.26%) male, aged between 5 and 75, all blood samples were collected at Akhmet Yassawi University (Kazakhstan) and analyzed at Gazi University (Turkey). Serum samples were tested for the presence of Rubella IgM and IgG antibodies using microparticulate chemiluminescent immunoassay (CMIA). Rubella nucleic acids were analyzed by real-time PCR.”

Results: “The overall seroprevalence rates for Rubella IgG antibodies were 290(75.2%) positive, 48(12.4%) negative, and 48(12.4%) equivocal, with a higher seroprevalence in men 151(41%) than in women 139(34.2%). The Rubella IgG seroprevalence showed significant differences between age groups: 33(78.5%) samples were from in the age group of 5–12 years; further, 27(60%), 92(80%), 109(73.6%) and 29(80.5%) were from age groups, 13–18, 19–40, 41–65 and 66–75 years, respectively. The Rubella IgM was found positive in one sample. Rubella RNA result was negative.”

Conclusions: “More than 77.9% of 19 to 40 year-old women (main reproductive age in Kazakhstan) are within acceptable immunity range. Level of immunity to rubella in women of reproductive age in this region appears insatisfactory. Rubella vaccine is in national immunization program since 2005. Individuals in 13-18 age group are not vaccinated, while 5-12 age group are vaccinated. Therefore, seroprevalence is higher in 5-12 age group than 13-18 age group.”
A RETROSPECTIVE COHORT STUDY OF TOXOPLASMOSIS INFECTION IN IRELAND, 2009-2018

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Aim: Congenital toxoplasmosis may result from an acute primary infection by the mother during pregnancy. It may cause stillbirth or miscarriage and serious, progressive complications in a child. The aim of this study is to evaluate the prevalence of toxoplasmosis infection from 2013-2018 and congenital toxoplasmosis from 2009-2018 in the National Virus Reference Laboratory (NVRL), Ireland.

Method: Results of serum samples submitted for IgA, IgM and IgG antibodies to toxoplasma were reviewed. Samples were sent to Toxoplasma Referral Laboratory where possible over the study period. Data was extracted from the Laboratory Information Management System (LIMS) from 01.01.2009 to 31.12.2018. A laboratory case of congenital infection was defined as IgA/IgM positive for 28 days of life or persistence of IgG in second year of life.

Results: Over the study period, a total of 32,302 samples were investigated for toxoplasma between 2013-2018 and 6644 samples were investigated for congenital toxoplasmosis between 2009-2018. 62 confirmed toxoplasma infections in adults between 2013-2018 and 7 confirmed congenital toxoplasma infections between 2009-2018 were identified; 3 in 2010, 1 in 2011, 1 in 2012, 1 in 2013 and 1 in 2018.

Conclusions: The incidence of acute toxoplasma infection appears relatively low in an Irish population. Seven children were serologically confirmed with congenital toxoplasmosis. Notably there were no congenital infections for a four-year period 2014-2017.
[P098] TRANSFER OF (HPIV-3) HN GENE TO TARGET EUKARYOTIC CELL TO EXPRESS APPROPRIATE RECOMBINANT PROTEIN TO PREPARE A SUBUNIT VACCINE

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Aim: Human Respirovirus-3 (HPIV-3) causes disease in Respiratory System. Prophylaxis against paramyxoviridae infections could gain via F and HN glycoproteins. There is not an appropriate Human Respirovirus-3 (HPIV-3) vaccine. The aim of project was amplification of HN gene and using these one for other steps including expression in eukaryotic cells and preparation of recombinant protein as a subunit vaccine against HPIV-3.

Method: HN containing plasmid was transformed to E. coli DH5α. Transformed bacteria was cultured in LB broth contain ampicillin as selection marker. After incubation in 37°, the recombinant vector was extracted. Primers was designed which include BamHI and HindIII restriction enzyme sequences and PCR was performed with pfu DNA polymerase enzyme. pcDNA3 eukaryotic vector was propagated and digested with BamHI and HindIII restriction enzymes. The PCR product was digested with the same enzymes. After ligation between digested vector and PCR product and gene transfer, the recombinant vector (HN/pcDNA3) was constructed and sequenced. Then, HN/pcDNA3 was transfected to COS7 cell line and gene expression was assessed.

Results: The synthetic HN gene was amplified via PCR and sub-cloned in pcDNA3 expression vector and confirmed with sequencing method. The HN protein 36 KD appeared on nitrocellulose membrane by using anti-His-tag antibodies. Conclusions: HN/pcDNA3 could expressed HN gene in COS7 cell line and possibly will be used in next project for animal model study. The result of western blot analysis was cleared recombinant HN was expressed in cell and may possibly purified and will use for assessment of immunological response in animal model.

Fig. 1. Western blot for the detection of the HN protein production. The weight of the protein is almost 36 kDa. Lines 2 and 3 confirmed expression of HN by anti-His antibody.
References:
Aim: The objective of this study was to compare measles RNA levels between naïve (i.e. primary) and breakthrough infections (i.e. infections in individuals with a history of vaccination) in cases from an outbreak of measles in Gothenburg, Sweden, where breakthrough infections were common. We also propose a fast-provisional classification of breakthrough infections.

Method: Medical records were reviewed, and real-time PCR-positive samples genotyped. Cases were classified as naïve, breakthrough or vaccine infections. We compared clinical symptoms and measles RNA cycle threshold (Ct) values between breakthrough and naïve infections.

Results: Sixteen of 28 confirmed cases of measles in this outbreak were breakthrough infections. A fast-provisional classification, based on previous history of measles vaccination and detectable levels of measles IgG in acute serum, correctly identified 14 of the 16 breakthrough infections, confirmed by IgG avidity testing. Measles viral load was significantly lower in nasopharyngeal samples from individuals with breakthrough compared with naïve infections (median Ct-values: 32 and 19, respectively, p < 0.0001). No onward transmission from breakthrough infections was identified.

Conclusions: Our results indicate that a high risk of onward transmission is limited to naïve infections. We propose a fast-provisional classification of breakthrough measles that can guide contact tracing in outbreak settings.

Prospective Study of Characteristics of Human Papillomavirus 6 and 11 Infection in Anogenital Warts and Corresponding Hair Follicles Over the Period of Two Years

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Aim: To determine whether in a cohort of men, prospectively followed-up every three months for up to two years, identical human papillomavirus (HPV) 6 and 11 viral variants were present in anogenital warts (AGW) and corresponding hair follicles and, additionally, to ascertain whether the identified viral variants were persistent and/or associated with greater likelihood of disease’s recurrence.

Method: At each sampling point, the present AGW and at least 5 hairs were obtained from 3 anogenital sites (scrotal, pubic and perianal) and eyebrows of each of the 32 male patients. DNA isolates were tested for the presence of HPV using HPV6/11 real-time PCR and GP5+/6+/68 PCR. HPV6 and 11 viral variants were determined based on the 960- and 208-bp representative regions for whole-genome-based phylogenetic clustering, using newly developed type-specific PCRs.

Results: All initial AGW harboured infections with single HPVs, with HPV6 (28/32; 87.5%), HPV11 (3/32; 9.4%) and HPV40 (1/32; 3.1%). All HPV6 (n=14) and HPV11 (n=1) viral variants, detected in hairs of a single patient, were identical in initial and all follow-up AGW samples. Based on the characteristics of HPV6 infection, patients were further divided into 3 groups (cleared AGW: n=15, persistent AGW: n=7, recurrent AGW: n=6). Taken together, three and two distinct HPV6 viral variants were present only in samples of patients with persistent infections and recurrent AGW, respectively.

Conclusions: Even though identical HPV6/11 viral variants persist in AGW/corresponding hairs for up to 2 years, the mechanisms of AGW recurrence still warrant further studies on a larger sample size.
DETERMINATION OF POTENTIAL CAUSATIVE ETIOLOGICAL HUMAN PAPILLOMAVIRUS TYPES IN HISTOLOGICALLY CONFIRMED COMMON WARTS, BASED ON ESTIMATED VIRAL LOADS

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Aim: To determine viral loads (VL) of alpha (HPV2/27/57) and mu (HPV1/63/204) human papillomaviruses (HPV) in tissue specimens of histologically confirmed cutaneous warts (CWs) with single and multiple HPV infections.

Method: Fifty-three and 71 DNA isolates with single and multiple HPVs were included, respectively, and analysed for VLs with type-specific quantitative multiplex HPV2/27/57 RT-PCR and three different type-specific quantitative mu PV RT-PCRs.

Results: The most frequent HPV types in CWs with single HPVs were HPV2/27/57 with overall prevalence of 77.3% (41/53), followed by HPV1 (3.8%, 2/53). In CWs with single HPV infection, median HPV2/27/57 DNA VLs were estimated to 3.2x10⁴, 2.8x10⁴ and 1.8x10⁴ viral copies/cell, respectively, and were significantly higher compared to HPV1 and HPV63 VL, which were estimated to 8.2x10⁻³ and 2.0x10⁻³ viral copies/cell, respectively. HPV2/27/57 VLs in CWs with multiple HPVs ranged from 6.0x10⁻⁴ to 3.6x10⁵ viral copies/cell, whereas VLs of HPV1/63/204 ranged from 7.0x10⁻⁴ to 1.6x10² viral copies/cell (generally lower than 1 viral copy/cell). HPV63 and HPV204 were not determined as causal agent due to constantly very low VL. In 10/53 and 15/71 CWs with single and multiple HPVs, respectively, etiological HPV could not be determined.

Conclusions: No significant differences in VLs of disease-causing HPV types in CWs with single and multiple HPVs were found. In majority of CWs with multiple HPVs, single dominant HPV type was present with very high VL, indicating etiological association.
HIGHLY SENSITIVE PARECHOVIRUS CODEHOP PCR AMPLIFICATION OF THE COMPLETE VP1 GENE FOR TYPING DIRECTLY FROM CLINICAL SPECIMENS AND CORRECT TYPING BASED ON PHYLOGENETIC CLUSTERING

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Aim: Human parechoviruses (HPeVs), particularly HPeV3, can cause severe neurological disease and neonatal sepsis in infants. HPeV3 lacks the receptor-binding motif Arginine-Glycine Aspartic Acid (RGD) and is proposed to use a different receptor associated with severe disease. In contrast, HPeV1, which contains the RGD motif, is associated with mild disease. Rapid characterization of the presence/absence of this motif is essential for understanding their epidemiology and differential disease profile.

Method: Current HPeV typing assays are based on partial capsid genes and often do not encompass the C-terminus where the RGD region is localized/absent. In addition, these assays lack sensitivity to enable characterization within low viral-load samples such as cerebral spinal fluid. Here, we developed a highly sensitive HPeV CODEHOP PCR, which enables typing of parechoviruses directly from clinical samples while generating a complete VP1 gene including the C-terminus.

Results: The assay was HPeV specific and had a sensitivity of 6.3 TCID50/ml for HPeV1 and 0.63 TCID50/ml for HPeV3. Analysis of the complete VP1 gene in comparison to partial VP1 fragments generated by previously published PCRs showed homologous clustering for most types. However, phylogenetic analysis of partial VP1 fragments showed incongruent typing based on the 75% homology classification rule. In particular, the strains designated as type 17 were found to be either type 3 or 4 by the (near) complete VP1 fragment.

Conclusions: While enabling sensitive characterization of HPeVs directly from clinical samples, the HPeV CODEHOP PCR enables characterization of RGD and non-RGD strains and correct HPeV-typing based on the complete VP1.
Aim: There is evidence that serological testing within 90 days of transfusion with blood products and/or IV immunoglobulin (IVIG) may give inaccurate results, with serology potentially reflecting the donor’s antibody status. The aim of the audit was to determine whether any patients at one NHS trust had had serology testing requested within 90 days post-transfusion.

Method: Patients who had received blood products and/or IV at a large university teaching hospital between 01/01/2017 and 31/12/2017 were identified. This list was compared a database of serological test requests, to identify patients who had had serology testing requested within 90 days post-transfusion. To determine frequency of incorrectly interpreted serology, CMV (cytomegalovirus) IgG samples were examined. Patients whose records demonstrated apparent changes to CMV serostatus were analysed in further detail.

Results: 13% (666/5100) of patients had serological testing within 90 days post-transfusion. 3053 tests were requested in total, with 45% of these requested in the first 7 days. 100 patients had CMV testing within the time period, with 17 samples meeting criteria for further analysis. Of these, 9 had serology that was likely secondary to passively transfused antibody, with 4 potentially clinically significant cases.

Conclusions: Approximately 1 in 7 patients who received blood products and/or IVIG had serological testing within 90 days post-transfusion. This represents a risk to patients if serology is interpreted incorrectly. This risk could be reduced through staff education and a warning on electronic test requesting systems. Further research is required to determine the frequency of the risks involved.
Aim: Rapid yet reliable tests for neutralizing antibodies (NAbs) would be desirable for determination of protective immunity and distinguishing serologically between infections of closely related pathogens. We have previously developed time-resolved Förster resonance energy transfer (TR-FRET)-based rapid immunoassays and demonstrated their performance in diagnosis of acute hantavirus disease. In this study, we explored the applicability of TR-FRET-based methods for rapid detection of NAbs.

Method: We set up two TR-FRET-based assays for antibodies against *Puumala orthohantavirus* (PUUV) Gn glycoprotein ectodomain (GnE): GnE-CFRET (competitive FRET immunoassay) and GnE-LFRET (protein L-based FRET immunoassay). We analyzed a 42-sample panel including 28 sera from patients with past PUUV infection using the two methods and quantified the corresponding neutralization titers with pFRNT (pseudotype focus reduction neutralization test).

Results: There was an evident correlation between GnE-LFRET signals and the neutralization titers. In detection of strongly neutralizing samples, GnE-LFRET was sensitive and specific. Likewise, with strongly neutralizing samples GnE-CFRET was specific, but not equally sensitive.

Conclusions: TR-FRET based assays seem promising in NAb detection, yet more work is required for the approach to be complete for field use. In the future, to improve detection of NAbs with TR-FRET-based approaches, multiple NAbs against various neutralization epitopes as well as different antigens could be employed.

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Aim: This study was to evaluate the performance of multiplex diagnostics kit by assay for respiratory viral pathogens* which can simultaneously detect multiple targets causing respiratory infection.

Method: Samples including aspirates, nasopharyngeal swab, bronchial lavages and throat swab from emergency room, newborn room, intense care unit, wards and out-patient department, were extracted using viral RNA mini kit**. The extracted nucleic acid was amplified and hybridized according to manufacturer's instructions, and finally analysed by instrument*, a flexible analyzer based on the principles of flow cytometry.

Results: Overall, the positive rates were 13.2% and 57.3% and co-infection rate were 4.9% and 21.6% for adults and children, respectively. Among 19 viral and bacterial targets included in assay for respiratory viral pathogens*, enterovirus/rhinovirus was mostly detected in both adults and children respiratory specimens, took up to more than 30% of all targets, and the second target detected were parainfluenza and Respiratory Syncytial Virus (RSV) for adults and children, respectively.

Conclusions: Multiplex diagnostics technique can greatly enhance testing efficiency by detecting multiple targets at the same time. Also, the technique shortens test turnaround time from days to hours compared to conventional culture methods, resulting in a better infectious diagnosis outcome.

* Luminex® xTAG® Respiratory Viral Panel Fast v2
**QIAGEN QIAamp*
[P107] IDENTIFICATION OF RHINO/ENTEROVIRUS MONOCLONAL ANTIBODIES THAT RECOGNIZE A BROAD RANGE OF RHINOVIRUSES

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\textbf{Aim:} Rhinoviruses are the most common causes of self-limited upper respiratory tract infections. They are routinely detected by RT-qPCR method, which targets conserved untranslated region of genome. Instead, conserved protein-encoding region has not been identified for development of broad range diagnostic antibodies. Consequently, there are not many rhinovirus antibodies with demonstrated broad range specificities in use. In this work, we analyzed several commercial “pan-rhino- and enterovirus” antibody reagents.

\textbf{Method:} HeLa cells were infected with repository of 99 cultivable serotypes representing species \textit{Rhinovirus} A and B. Cells were fixed and stained with pan-rhino/enterovirus antibodies. Antibody against dsRNA was used as positive infection control. Infected cells were imaged using immunofluorescence microscope (EVOS FL AUTO). Raw images were further processed using ImageJ software, and positivity was recorded.

\textbf{Results:} “Pan-rhinovirus” antibodies generated against recombinant capsid protein detected 55 out of 99 serotypes (18 strong positives), while “pan-enterovirus” antibodies generated against recombinant capsid protein and intact enterovirus were positive against 67 (11 bright-fluorescing) and 15 out of 99 serotypes, respectively.

\textbf{Conclusions:} Results indicated that some antibodies may be useful in cellular studies of infection by non-conventional RVs (other than RV-A2, -B14 and -A16). The data also indicate that both pan-rhino- and pan-enterovirus antibodies bind to conserved epitopes between several virus types. Identification of these epitopes would enable development of high-affinity and broad-range diagnostic RV/EV-antibodies via antibody library panning and affinity maturation using phage display technology. Such antibodies would be useful in development of rapid point-of-care assays, to be used in test systems such as mariPOC.
PERFORMANCE EVALUATION OF THE NOVEL ALTOSTAR HCV RT-PCR KIT ON THE FULLY AUTOMATED ALTOSTAR PLATFORM

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Aim:

For patients chronically infected with hepatitis C virus (HCV) RNA viral load monitoring is recommended by current guidelines to determine the efficacy of treatment. The goal of therapy is to reach a sustained virologic response, which is defined as undetectable HCV RNA plasma/serum concentration using a sensitive HCV RNA quantitation assay with a lower limit of quantification of ≤25 IU/ml. The AltoStar HCV RT-PCR Kit 1.5 is a novel assay, which recently received CE mark. The objective of this study was to evaluate the performance of the AltoStar HCV RT-PCR Kit 1.5 on the automated AltoStar System.

Method:

We used the 5th WHO International Standard for HCV NAT (HCV genotype 1a) to determine the limit of detection (LoD). Probit analysis was performed to calculate the LoD. The clinical performance of the AltoStar system was compared to the Abbott RealTime HCV assay on the Abbott m2000 system. In total, 480 samples from HCV-infected patients were used. We assessed diagnostic sensitivity and specificity and compared quantitative results by linear regression analysis and Bland-Altman Plot.

Results:

The LoD of the AltoStar for the detection of HCV genotypes 1a, 1b and 2 to 6 in EDTA plasma was 11.1 IU/ml (95% confidence interval(CI): 7.8 – 18.5 IU/ml). The analytical specificity was 100% as assessed on 100 HCV RNA negative samples.

Conclusions:

The AltoStar HCV RT-PCR Kit demonstrated an analytical and diagnostic performance comparable to that of a currently market-leading HCV assays. It may aid in clinical decision making of HCV infected patients.
[P110] ADAPTATION OF LABORATORY DEVELOPED TESTS TO THE OPEN ACCESS SYSTEM

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Introduction: This new molecular testing instrument* is an easy to use, molecular based high throughput system with continuous loading. Open Access software is available, which makes it possible to run CE-IVD assays together with laboratory-developed tests (LDT), increasing efficiency.

Aim: To investigate whether LDT assays can easily be adapted to new molecular testing instrument* assays.

Method: Published Enterovirus (EV), Bocavirus (HBoV) and Coronavirus (HCoV) internally controlled (IC) LDT PCR assays using Roche FLOW were adapted to the molecular testing instrument* Open Access protocol. Adaptation included shortening cDNA synthesis and reducing the total number of PCR cycles. Two multiplex assays were analyzed: EV/OC43/IC and HBoV/229e/ NL63/IC. Analytical sensitivity and specificity were assessed using positive virus culture stocks. Retrospective assessment of clinical performance included 109 stored (-80°C) samples collected between January 2005 and December 2016.

Results: Analytical sensitivity was generally slightly lower for the Open Access assays. Clinical specificity and sensitivity were between 89.3%-96.6% and 100%, respectively. Discrepant results were found in 6 (5.5%) samples, which tested positive in LDT (Ct> 31.4) and negative in molecular testing instrument*.

Conclusions: LDT assays can easily be adapted to the molecular testing instrument* Open Access protocol and performed simultaneously with CE-IVD assays, albeit with a slightly less clinical sensitivity compared to the LDT.

* Panther Fusion®
EVALUATION OF GENOMERA® CDX SYSTEM FOR INFLUENZA AND RSV INFECTIONS

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Aim: Performances comparison [Concordances(κ), sensitivity(Se), specificity(Sp)] of RT-qPCR assays detecting major viral respiratory pathogens on the GenomEra®CDX system, using the GenomEra®FluA/B+RSV assay (Influenzavirus (IAV/IBV), or Respiratory Syncytial virus (RSV)) to our standard of care process (Allplex Respiratory; Seegene).

Method: Prospective assays on fresh samples (n = 299; 116 nasal swabs (NS), 129 combined nasal and throat swabs (NTS) and 54 nasopharyngeal aspirates (NPA)) collected for 2018-2019 winter season, from patients hospitalized in the University Hospital of Poitiers, France. Samples with discrepant results were tested with a 3rd method: a real time PCR method (R-gene, Biomérieux®). Our referential consider concordance of ≥2 molecular testing specific for a specific viral target (including the result of the third method if needed). Inclusivity, cross-reactivity and reproducibility testing were associated to analyses on clinical samples.

Results: Performances could be obtained for each viral target (no IBV-positive sample during the epidemics). On NS, Se, Sp, and κ were: i) 100%, 95% and 0.89 for IAV; ii) undetermined, 97%, and undetermined for IBV; iii) 100%, 100%, and 0.91 for RSV. On NTS, performances were: i) 100%, 99% and 0.96 for IAV; ii) undetermined, 98% and undetermined for IBV; iii) 95%, 100% and 0.92 for RSV. On NPA performances were: i) 86%, 100% and 0.91 for IAV; ii) undetermined, 100% and undetermined for IBV; iii) 97%, 100% and 0.92 for RSV.

Conclusions: With 1 false negative and 5 false positive (including one IBV) results, performances for Influenza detection are very good but remain perfectible. With 1 false negative result, performances for RSV detection are very impressive.
IN SILICO EVALUATION OF THE AFFINITY OF ANTIVIRALS AGAINST HCV NS5 PROTEIN IN ZIKA VIRUS

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Aim: The viruses of flaviviridae family cause several diseases like hepatitis C (HCV) and others like zika (ZIKV) and dengue fever (DENV). According to the world health organization (WHO), HCV causes more than 71 million of infections worldwide. However, there are treatments for the disease using drugs designed against the NS5 protein of the virus (ARN polymerase). On the other hand, Zika virus continues to spread in different parts of the world and the recent epidemic of ZIKV in the Americas has been associated with fetal and neurological complications. Consequently, antivirals capable of inhibiting the replication of the Zika virus are necessary, because vaccines are not yet available to prevent the disease. For this reason and knowing that structure of flaviviridae family NS5 protein reveals a conserved domain conformation, we probed computationally the drugs designed against HCV on ZIKV.

Method: We use molecular dynamics and molecular docking to anchor the antivirals of HCV (Dasabuvir (ABT-333), ABT-072, Filibuvir, GS-9669, Lomibuvir, Nesbuvir, and Setrobuvir) on ZIKV.

Results: The bioinformatic prediction was possible to determine a chemical interaction the antivirals with the active site of the NS5 polymerase of Zika virus.

Conclusions: In this way, antivirals tested in this study are promising to move on to in vitro analysis models and could be use in the future as treatment for ZIKV and prevent the spread of the disease.
COMPARATIVE EVALUATION OF THE NEW HSV1&2 VZV R-GENE® KIT AND A REAL-TIME PCR LABORATORY-DEVELOPED TEST (LDT) FOR THE DETECTION AND QUANTIFICATION OF VARICELLA-ZOSTER VIRUS (VZV) GENOME IN CLINICAL SAMPLES

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Aim: ” VZV is a common pathogen responsible for mucocutaneous, cerebral, ocular and disseminated infections. Rapid and accurate laboratory diagnosis of VZV infections in a large variety of specimens is essential for optimal clinical and therapeutic management. The objective of this study was to evaluate the new HSV1&2 VZV R-GENE® kit (reference 69-014B under development, ARGENE®, BIOMERIEUX) in comparison to our routine real-time PCR LDT for the detection and quantification of VZV genome”

Method: ” A total of 70 VZV positive and negative samples were included: whole bloods (16), mucocutaneous swabs (24), cerebrospinal fluids (CSFs) from patients (10), VZV-negative CSFs spiked with different concentrations of VZV ATCC strains (10), QCMD 2018 samples (10). Nucleic acid extraction was performed using EMAG® (BIOMERIEUX), assay set-up using ESTREAM® (BIOMERIEUX), DNA amplification using LightCycler®480 (Roche Diagnostics). Both assays were performed on the same day with the same nucleic acid eluate, previously stored at -80°C. R-GENE® assay was performed according to the manufacturer’s recommendations; LDT was performed as previously published (Burrel et al., J Virol Methods, 2012)”

Results: “No PCR inhibition was observed. The concordance between R-GENE® kit and LDT was 100%. The comparison of the 64 positive samples showed an excellent correlation between the VZV loads measured by both techniques (Spearman’s coefficient of rank correlation = 0.985; P<0.0001) with an average bias of -0.28 log copies/mL (Bland-Altman test)”

Conclusions: ” HSV1&2 VZV R-GENE® kit constitutes a suitable method for the detection and quantification of VZV genome in clinical samples in a routine laboratory setting”
[P114] EVALUATION OF THE NEW PCR KITS FOR THE QUANTIFICATION OF HERPES SIMPLEX VIRUS 1 (HSV-1) GENOME IN BRONCHOALVEOLAR LAVAGE (BAL) FROM PATIENTS WITH BRONCHOPNEUMONITIS (BPN)

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Aim: “In immunocompetent patients with prolonged mechanical ventilation, HSV-1 may reactivate in the oropharynx and contaminate gradually the lower respiratory tract, leading to BPN. We previously showed that HSV-1 load in BAL above 80.000 copies/10⁶ cells was predictive of the onset of BPN in patients from intensive care unit (Luyt et al., AJRCCM, 2007). The objective was to evaluate the new HSV1&2 VZV R-GENE® (69-014B under development) and the CELL control R-GENE® kits (ARGENE®, BIOMERIEUX) in comparison to our routine laboratory-developed techniques (LDTs) for the quantification of HSV-1 and cells in BALs”

Method: “Fifty sequential BALs from 18 different patients were analyzed. Nucleic acid extraction was performed using EMAG® (BIOMERIEUX), assay set-up using ESTREAM® (BIOMERIEUX), DNA amplification using LightCycler® 480 (Roche Diagnostics). All assays were performed on the same day with the same nucleic acid eluate, previously stored at -80°C. Commercial assays were performed according to the manufacturer’s recommendations; LDTs were performed as previously published (Burrel et al., J Virol Methods, 2012)”

Results: “The concordance between R-GENE® techniques and LDTs was 94%. The comparison of the 33 positive BALs showed a good correlation between HSV-1 loads measured by both techniques (Spearman’s coefficient of rank correlation = 0.971; P<0.0001) with an average bias of -0.75 log copies/10⁶ cells (Bland-Altman test). Kinetics of HSV-1 loads were similar with both techniques”

Conclusions: “HSV1&2 VZV R-GENE® and CELL control R-GENE® kits allow the accurate quantification of HSV-1 load in BALs in a routine laboratory setting for the diagnosis of HSV-1 BPN”
EVALUATION OF THE COBAS PLASMA SEPARATION CARD AS A SAMPLE TYPE FOR HCV VIRAL LOAD QUANTIFICATION ON THE COBAS 6800/8800 SYSTEMS.

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Aim: Evaluate the cobas Plasma Separation Card (PSC) as an alternative sample type for HCV viral load on the cobas 6800/8800 Systems.

Methods: Matched PSC and plasma were collected from 50 chronic HCV subjects. Plasma and one PSC were frozen at -10°C while one PSC was kept at room temperature for 30 days before testing. Plasma testing was performed following the manufacturer’s instructions while 1 spot of each PSC was eluted with 950µL of cobas SPER in a cryo tube and submitted to heating & shaking in a thermomixer for 10 min at 56°C and 1100 rpm before testing using the cobas HCV test on the cobas 6800 System using the plasma option as sample type. Results from the PSC were plotted against the plasma results. A panel with HCV secondary standard traceable to the WHO standard spiked in whole blood was used to estimate the LOD on PSC.

Results: The limit of detection of the cobas HCV Test using the PSC was 866 IU/mL (95% CI, 698–1,153 IU/mL). The mean titer differences between frozen PSC samples and EDTA plasma samples, and between ambient PSC samples and EDTA plasma samples was -1.63 log10 IU/mL (95% CI -1.70– -1.57) for both comparisons. Correlation between the EDTA-plasma and PSC was linear (slope=1.04, intercept=-1.84 [ambient], R²=0.94 and (slope=1.01, intercept=-1.71, R²=0.94 [frozen]).

Conclusions: The results demonstrate good correlation between PSC and EDTA plasma samples with sensitivity <1,000 IU/mL. Use of PSC for sample collection could offer increased access to HCV testing.
MULTI CENTER PERFORMANCE EVALUATION OF AN EBV DNA QUANTITATIVE PROTOTYPE TEST FOR THE COBAS 6800/8800 SYSTEMS

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Aim: To evaluate analytical and clinical performance of a quantitative EBV DNA prototype test* for the cobas 6800/8800 Systems.

Methods: Panels and clinical materials were measured with the cobas EBV prototype test (three study sites: Graz, Hamburg, Paris) and with CE approved Epstein-Barr Virus (EBV) tests**: A 4 levels panel (4 replicates) ranging from 1E2 to 1E5 IU/mL were prepared using 1st WHO International Standard for EBV. Clinical samples (n=30) positive for EBV were diluted in EBV negative human plasma. The test accuracy was calculated as delta observed minus expected [log IU/mL] for panels and clinical samples. The WHO panel was further used to calculate a correction factor to IU/mL for the tests that report results in copies per milliliter.

Results: The accuracy, in log IU/mL, to WHO varied from -0.04 to 0.06 (Graz), and -0.02 to 0.10 (Hamburg) on the EBV prototype*, -0.26 to 0.03 on the**, and -0.15 – 0.09 on the test**. Clinical sample testing revealed the mean log IU/mL difference observed with 30 samples tested in two sites on the cobas EBV prototype was 0.07. The mean difference between the EBV prototype test* and CE approved Epstein-Barr Virus (EBV) tests**: was -0.23, -0.14, -0.88, and 0.12 log IU/mL, respectively.

Conclusions: The cobas EBV prototype test was accurate, reproducible and demonstrated high agreement with the EBV WHO standard. However, higher variability was observed with clinical specimens across different assays.

*cobas® EBV prototype
** bioMerieux R-Gene (Graz); Qiagen Artus and Altona RealStar (Hamburg); Abbott Realtime (Paris)
[P117] DUAL CENTER PERFORMANCE EVALUATION OF A BKV DNA QUANTITATIVE PROTOTYPE TEST FOR THE COBAS 6800/8800 SYSTEMS

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Aim: To evaluate analytical and clinical performance of a BKV DNA quantitative prototype test (cobas BKV prototype) for the cobas 6800/8800 Systems.

Methods: Panels and clinical materials were measured with the cobas BKV prototype test (two study sites: Graz, Hamburg) and with CE approved tests: (bioMerieux R-Gene (Graz); Qiagen Artus and Altona Diagnostics RealStar (Hamburg). Two 4 levels panels (4 replicates) ranging from 1E2 to 1E5 IU/ml were prepared using 1st WHO International Standard for BK Virus (BKV) and NIST standard Reference Material (R2365). BKV positive clinical samples (n=30) were diluted in BKV negative human plasma. The test accuracy was calculated as delta observed minus expected [log IU/ml] for panels and clinical samples. The WHO panel was further used to calculate a correction factor to IU/ml for the tests that report results in copies per milliliter.

Results: The accuracy, in log IU/ml, to WHO/NIST varied from -0.08/-0.04 to 0.05/0.11 (Graz), and -0.07/0.08 to 0.03/0.30 (Hamburg) on the cobas BKV prototype and, 0.20/0.03 to 0.31/0.33 on the RealStar test. Clinical sample testing revealed the mean log IU/ml difference observed with 30 samples tested in two sites on the cobas BKV prototype was 0.07. The mean difference between the cobas BKV prototype test and R-Gene, Artus, or RealStar was -0.57, 0.14, and -0.23 log IU/ml, respectively.

Conclusions: The cobas BKV prototype test was accurate, reproducible and demonstrated high agreement with BKV WHO and NIST standards, and good agreement in a first set of clinical samples compared to CE tests.
Aim: Nucleic acid-targeted amplifications are gold standards for detecting pathogens, delivering high sensitivity without host responses. While PCR is very successful in this capacity, many seek isothermal replacements to simplify instrumentation and (hopefully) enable amplification without sample preparation. Unfortunately, primers in isothermal amplifications do not always behave as expected, due to oligonucleotide-oligonucleotide interactions that create low melting primer-dimers, off-target priming, and amplification "monsters". Further, sample preparation easily comes to dominate assay cost. We mitigate these problems using innovations in synthetic biology and innovative sample collection methods.

Method: Two new kinds of DNA, artificially expanded genetic information systems (AEGIS) and self-avoiding molecular recognition systems (SAMRS), were incorporated into a variety of isothermal amplification systems. These were coupled to a sample-extraction architecture where arbovirus vectors themselves provide the sample on a DNA/RNA capture matrix. This exploited a support covered with quaternary ammonium groups (Q-paper) and baited with honey from which mosquitoes deposit saliva containing arboviruses after being attracted by a novel CO₂ generator.

Results: Isothermal approaches were shown to directly detect, in multiplexed form, 10-100 copies of dengue, chikungunya, and Zika viruses in low multiplexed isothermal amplifications. Similarly, 50 copies of HPV DNA and 50-100 copies of tick-borne pathogens were isothermally detected within 30-45 minutes. These were compared to LODs from assays using urine, plasma, crushed mosquito or tick carcasses as samples.

Conclusions: These results deliver low cost platforms to survey environments for active arboviral transmission potential with applications to human diagnostics in low resource environments.

Funded by NIAID-1R21AI128188-01, NIAID-5R21AI137768-02, and FDACS-024376.
Aim: PCR is widely used to detect target DNA and RNA (with reverse transcriptase) with defined target sequences. However, multiplexed PCR is desired to spread the costs of PCR over multiple targets. Unfortunately, primers in multiplexed PCR do not always behave as expected from singleplexed PCR, giving artifacts that often collapse pre-established assays upon addition of new targets. We have mitigated this using two innovations in DNA chemistry, the first being pseudo-complementary DNA built into target-specific primers, and orthogonal DNA built into external primers in a nested PCR architecture.

Method: A panel with 42 primers incorporating pseudo-complementarity and orthogonality was created to detect 21 different arboviruses, with the detection system exploiting transliteration, another innovation that allows orthogonal DNA to “talk to” standard DNA. Signal was generated using bead-based hybridization architecture. Then, with the appearance of chikungunya and Zika in Florida, the multiplex was expanded to 22 and 23 targets.

Results: The pseudo-complementarity and orthogonality of the assay chemistry allowed the multiplex to be created without careful design to avoid primer-dimers, off-target hybridization, and other artifacts that often make multiplexed PCR challenging. Further, the multiplex effortlessly survived expansion of the multiplex, twice. These two features of the assay chemistry also allowed uniform detection of targets, despite the highly multiplexed design. Rules allow the design of these systems, which may be coupled to biversal nucleotides to manage rapid RNA viral sequence divergence.

Conclusions: These reagents offer users access to highly sensitive highly multiplexed PCR with highly uniform amplicon detection.

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RAPID AND COST-EFFECTIVE ROUTINE VIRAL GENOTYPING USING NANOPORE SEQUENCING

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Aim: Viral genotyping is an important tool to guide and monitor treatment decisions, support and evaluate (hospital) infection control measures, and contribute to public health surveillance of a broad range of diseases including viral hepatitis, enteroviruses and HIV. Currently, routine genotyping is mostly performed using targeted amplification and subsequent Sanger sequencing, with target choice and performance characteristics tuned to specific use cases. The fast development of deep sequencing platforms does offer potential for merging clinical and public health applications into a single combined application, thus potentially cost-saving when used in clinical laboratories with sufficient throughput for pooling of analyses.

Method: We have developed a rapid multiplexing and library preparation protocol using real-time nanopore sequencing to determine the genotypes of multiple viral targets. Thus far, these viral targets include HBV, HCV and measles virus. One nanopore run allows for simultaneous sequencing of multiple viral targets.

Results: Viruses from various species, genotypes and viral loads were rapidly nanopore sequenced, demultiplexed and subtyped. All samples were confirmed by Sanger sequencing as the gold standard. The reported high error rate associated with the nanopore sequencing chemistry was resolved by adequate coverage and consensus sequencing.

Conclusions: We were able to accurately subtype a variety of viral species. Furthermore, multiple samples from different virus species were successfully combined, sequenced, and demultiplexed after one sequencing run. This allowed us to rapidly genotype viruses. Further efforts will determine the feasibility of nanopore sequencing in antiviral resistance testing.
LONGITUDINAL ANALYSIS OF HEPATITIS C IGG AVIDITY CAN DISTINGUISH RECENT FROM PAST HEPATITIS C INFECTION.

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Aim: Acute HCV infection is generally asymptomatic and can remain undiagnosed, therefore the need for early and accurate diagnosis is important. The aim of this study was to develop an HCV IgG avidity assay by modifying an HCV enzyme linked immunoassay to include incubation with putative dissociation buffers to disrupt the low avidity antibodies.

Method: Sera from HCV infected patients comprised clinically acute (n=15) and chronically infected (n=29) patients. Longitudinal assessment of HCV IgG avidity was performed in a subset of patients where seroconversion was observed.

Results: A significant correlation of the HCV IgG avidity indices was observed using either urea (6M) or citrate buffer (0.1M) as dissociation agents (r=0.74, p<0.0001). Significant differences in avidity were observed between acute and non-acute samples using citrate (mean ± SE: 25.78 ± 7.5 vs 60.53 ± 4.7, p<0.0001) and urea (58.46 ± 9.06 vs 87.45 ± 2.63, p<0.0001). Using an avidity index cut off of 30%, citrate correctly identified 73% of samples as recent compared to 27% when urea was used. Longitudinal analysis of 15 samples obtained from 5 recently HCV infected patients demonstrated increasing IgG avidity with time since onset of infection (r=0.65, p<0.02). Furthermore, delayed maturation of the HCV IgG avidity response was observed in HCV infected patients who were co-infected with HIV.

Conclusions: Our findings show potential for the development of an HCV IgG avidity assay which may be useful to initiate early treatment, prevent liver damage and to estimate HCV infection incidence.
[P122] ANALYSIS OF THE VARIATION IN HA GENE SEQUENCE AND EVALUATION OF THE SHEDDING OF H9N2 VIRUS USING REAL-TIME RT-PCR IN SPF CHICKENS

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Aim: In this study, we evaluated the shedding of Iranian strains influenza virus (H9N2) in the oropharyngeal and cloacal routes of the SPF chicks by using the RRT-PCR molecular technique. Furthermore, the genetic changes of the cleavage site in the above-mentioned virus were analyzed with other influenza viruses and phylogeny study was done.

Method: In the present study, chickens were inoculated with LPAIV (A/chicken/Iran/ZMT-101/1998(H9N2)). OP and CL swabs were collected from chickens from 1 to 10 days after the inoculation. The rate of viral shedding was measured within 10 days by the RRT-PCR (Real-Time Reverse Transcriptase Polymerase Chain Reaction). The sequence analysis and phylogenetic study of the samples were performed by comparing each isolate with the other H9N2 isolates in the gene bank.

Results: The results showed that the main route of shedding for LPAIV was in OP areas (p<0.05). Both isolates had an R-S-S-R sequence at the cleavage site of HA gene and contained glutamine (Q) amino acid at position 226 of the HA receptor-binding site. Phylogenetic analysis indicated that both isolates belonged to the Eurasian clade.

Conclusions: The main route of the virus replication is in the areas in which trypsin-like enzymes are present, through the oropharyngeal route was found to be the main replication site for the influenza virus. In phylogenetic studies, two D1 and D7 isolates were revealed that these isolates belonged to the Eurasian subcategory. The phylogenic analysis in this study indicated that D1 and D7 isolates had a similarity of 99%.
COMPARISON OF TWO POINT-OF-CARE INFLUENZA DETECTION METHODS IN THE FLU-SEASON 2018 AND 2019

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Aim: The purpose of this study was to evaluate the performance of two rapid molecular detection methods for Influenza virus – Simplexa\textsuperscript{TM} and Solana\textsuperscript{®} - as compared to a standard real-time-RT-PCR technology.

Method: For both direct amplification kits, Solana\textsuperscript{®}Influenza-A+B-Assay and Simplexa\textsuperscript{TM}FluA/B&RSV-Direct-Kit, dry respiratory swabs were saturated and could then be directly used. Simplexa\textsuperscript{TM} has a short RNA extraction and real-time-RT-PCR amplification without additional hands-on-time. Solana\textsuperscript{®}-Assay consists of two major steps with a short specimen preparation and an isothermal reverse-transcriptase-helicase dependent amplification. The results of these methods were compared with our standard technology RealStar\textsuperscript{®}Influenza-RT-PCR-Kits. In this case, a previous nucleic acid extraction was performed with Biomerieux EasyMag.

Results: In total 25 of the 50 samples were positive for Influenza-B using the RealStar\textsuperscript{®}-Assay. 20 swabs were positive with Simplexa\textsuperscript{TM} and 21 using Solana\textsuperscript{®}PCR. Comparing the results of Influenza-A we had 9 positive patients using the RealStar\textsuperscript{®}Kit, 8 positive using Simplexa\textsuperscript{TM} and 6 using Solana\textsuperscript{®}. In one case Influenza-A could only be detected by Simplexa\textsuperscript{TM} and another case only by Solana\textsuperscript{®}, whereas RealStar\textsuperscript{®} was negative.

Conclusions: Due to the genetic diversity of Influenza not every PCR may detect all different strains equally, which may explain some discrepancies. The RealStar\textsuperscript{®}technology seems to be slightly more sensitive probably due to a sample volume of 200µl used for nucleic acid extraction, whereas 50µl are sufficient for both Simplexa\textsuperscript{TM} and Solana\textsuperscript{®}technology. For epidemiological purposes the differentiation between Influenza-A/B/H1N1nv, which is done by RealStar\textsuperscript{®} is helpful, however, the detection of RSV by Simplexa\textsuperscript{TM}, may provide advantages in pediatric settings.
CYTOMEGALOVIRUS RETINITIS: APPLICATION OF A METAGENOMIC DEEP SEQUENCING APPROACH TO INVESTIGATE THE VIRAL REPLICATION STATE AND TO DETECT DRUG RESISTANCE MUTATIONS

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Aim: “The diagnosis of cytomegalovirus (CMV) retinitis relies on detailed funduscopic examination and detection of CMV DNA in ocular fluid by real-time PCR. CMV retinitis is typically treated with systemic ganciclovir or foscarnet with or without intraocular antiviral injections. Detection of drug resistance mutations (DRMs) within viral phosphotransferase (UL97) and/or DNA polymerase (UL54) has been associated with recurrent or progressive CMV retinitis despite therapy. We report a case of a 19-year-old hematopoietic stem cell transplant recipient experiencing bilateral CMV retinitis. Despite successive regimen of antivirals, persisting CMV DNA was detected 7 months after the initial episode, raising the suspicion of CMV resistance to antivirals. Several attempts to detect DRMs using classical Sanger sequencing resulted in failure even though high viral loads were measured by real-time PCR.”

Method: “As we hypothesized that long-range PCR amplification failure was due to fragmented CMV DNA within ocular compartment, we decided to use shotgun metagenomic sequencing (SMg) on whole-genom sequencing solution*. Our approach included genome reconstruction, variant calling (detection of DRMs), and transcriptomic analysis (detection of viral transcripts) using software**.”

Results: “While no DRMs were detected within UL97 and UL54 genes, our results evidenced the detection of viral transcripts within both ocular compartments.”

Conclusions: “Our results seem to tip the scales in favor of SMg for the analysis of ocular specimens in such clinical situations. Further studies are required to define the clinical significance of viral mRNA detection (viral replication state or remnant signals?) within the ocular compartment using SMg technology.”

* NextSeq®500 platform (Illumina)
** MetaMIC ® software
MINION SEQUENCING AND STRAIN SEPARATION OF WHOLE HUMAN CYTOMEGALOVIRUS GENOMES

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**Aim:** “To apply MinION sequencing to human cytomegalovirus (HCMV; 236 kbp) and implement a pipeline for analysing strains from high-titre clinical samples.”

**Method:** “Three previously sequenced laboratory strains of HCMV (Merlin, AF1 and U11) were grown to high titre in human foetal foreskin fibroblast cells, and DNA was extracted from cell-released virus. Sequencing libraries were prepared using an SQK-LSK109 kit and analysed on MinION flowcells (FLO-106). Basecalling was performed using albacore and then uppy. Consensus sequences were determined by *de novo* assembly using Canu or by BLAST analysis with MiniMap2. Merlin, AF1, U11 and a mixture of AF1 and U11 (AF1+U11) were analysed.”

**Results:** “The Merlin, AF1, U11 and AF1+U11 data included 3, 11, 30 and 161 reads of >200 kb, respectively, with the longest ranging from 234,848 to 299,468 bp. The top BLAST hits obtained using the longest reads matched the cognate strains and successfully separated the components of the mixture. The *de novo* assembled Merlin consensus sequence was >99% accurate, and the MiniMap2-generated consensus sequences were similarly accurate. The novel program minion_genotyper correctly identified the strains present in the mixture by analysing a set of hypervariable genes. Mummerplots of the longest reads identified the four anticipated genome isomers.”

**Conclusions:** “MinION sequencing is suitable for accurate analysis of single and mixed strains of HCMV from high-titre cultured samples without target-specific enrichment. Its high error rate did not adversely affect strain identification, probably as a result of high coverage depth. We plan to adapt this pipeline for sequencing high-titre clinical samples.”
Aim: Monitoring the viral load is an essential part of the management of patients chronically infected with hepatitis B virus (HBV). Currently, commercial HBV viral load assays are generally performed on high-throughput platforms for batch-wise testing of plasma samples with often long turnaround times. We therefore tested the new Cepheid Xpert® HBV Viral Load Assay for random access, rapid viral load testing to provide rapid input to clinical decision making.

Method: Stored plasma samples of 106 patients were selected, based on known HBV viral loads by the Cobas® Ampliprep/Taqman or Cobas® 4800, to compare with the Cepheid Xpert® HBV Viral Load Assay. Genotypes for 52 samples were known and included A, B, C, D, E and G. HBV DNA load ranged from <20 to 5*10^8 IU/mL; 32 samples were previously tested negative for HBV DNA. The acceptance criteria for inter-assay difference were set at 0.5 log and concordance at >95%. The correlation between the viral load results of both assays was determined by calculating the Pearson correlation coefficient (r). The level of concordance was assessed using the Bland-Altman analysis.

Results: Four samples exceeded the 0.5 log difference, setting the concordance at 96%. High correlation of HBV viral load across all genotypes were found (Figure, Pearson correlation r =0.997). Turnaround time using the GeneXpert® System was approximately 1 hour.

Conclusions: High agreement in HBV viral loads were observed between routine laboratory tests and the Xpert® HBV Viral Load Assay, enabling rapid, accurate virological assessment of HBV infected patients.
DETECTION OF CONJUNCTIVAL PATHOGENS EMPLOYING THE FTD EYE SYNDROMIC REAL-TIME PCR ASSAY

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**Aim:** The etiology of external eye infections is often misdiagnosed. In this study, we applied an eye panel syndromic real-time PCR assay to identify pathogens causing conjunctivitis.

**Method:** Conjunctival swabs were prospectively collected from patients of the emergency service of a tertiary academic center presenting with keratitis or conjunctivitis suggestive for HSV, Chlamydia or adenovirus infection. Specimens were collected using the Purflock® Ultra swab (Medical Wire, UK), processed in the fully automated QIAsymphony® SP platform (QIAGEN, Germany) and ocular pathogens were detected using the FTD Eye multiplex real-time PCR assay (Fast Track Diagnostics - a Siemens Healthineers company, Esch-sur-Alzette, Luxembourg) using a Rotor Gene Q Real-Time thermal cycler.

**Results:** A total of 29 samples obtained from 23 patients (10 males, 13 females, age 22-75 y.o.) with presumed acute conjunctivitis were tested for Herpes Simplex Virus Type-1 (HSV-1), Herpes Simplex Virus Type-2 (HSV-2) varicella-zoster virus (VZV), human adenoviruses and Chlamydia trachomatis. Adenovirus was detected in 7 out of 23 patients (30.4%). All adenovirus-positive samples confirmed the presumed clinical diagnosis for an adenoviral. Interestingly, adenovirus DNA was found in all six patients whose swabs were obtained from both the left and the right eye. HSV-2 was detected in one sample.

**Conclusions:** The FTD Eye multiplex real-time PCR assay can be reliably employed for the detection of pathogens causing conjunctivitis with an acceptable rate of agreement between presumed diagnosis and identification of the causing microorganism. The prevalence pattern indicates that among all pathogens tested, adenovirus has a major role in viral conjunctivitis.
[P128] VALIDATION OF TECHNOLOGY FOR THE DETECTION OF FLAVIVIRUS/ALPHAVIRUS AND ITS APPLICATION TO THE 2016/2017 VANUATU DENGUE OUTBREAK

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Aim: “The family flaviviridae and alphaviridae contain a diverse group of pathogens that cause significant morbidity and mortality worldwide. Few real-time PCR assays are able to detect the presence of all members of these families using single primer and probe sets. We have developed a novel chemistry, which simplifies the viral nucleic acids allowing the design of RT-PCR assays capable of pan-family identification. We sought to optimise the assay prior to determining its utility during the 2016/17 Vanuatu dengue-2 outbreak.”

Method: “Before testing on clinical samples synthetic constructs, viral nucleic acids, viral particles spiked into control serum, and characterised panels were used to determine the specificity and sensitivity of the assays.”

Results: “Synthetic constructs showed sensitivities of the pan-flavivirus and pan-alphavirus assay in the range of 10–25 copies per reaction. LLOD studies using virus particles demonstrated sensitivity of 1–8 copies. No cross reactivity was observed with a number of commonly encountered viral strains. Proficiency panels showed 100% concordance with the expected results. Testing during the 2016–2017 Vanuatu dengue-2 outbreak produced 116 positives from a total of 187 suspected dengue samples tested. Additionally, certain islands contained more cases than would be expected by population density alone.”

Conclusions: “The pan-viral screening assays described utilise a novel chemistry and are shown to provide a sensitive and specific method to screen and thereafter speciate viruses in clinical samples. The assays performed well in an outbreak situation and can be used to detect positive clinical samples in approximately 3 hours 30 minutes.”
Aim: Therapy monitoring of chronic infections with hepatitis B virus (HBV) requires a reliable detection of resistance against antiviral drugs. Since 2016, INSTAND e.V. has performed three runs of the External Quality Assessment (EQA) scheme for genotypic resistance determination in cooperation with the National Reference Centre (NRZ) for HBV and HDV, Giessen.

Method: The laboratories participating in these EQA schemes (up to 33) received a panel of four different samples with linearized plasmids* containing HBV DNA sequences carrying different resistance-associated mutations. The laboratories were requested to report the following results and conclusions: (i) sequence of the HBV polymerase gene, (ii) HBV genotype, (iii) resistance-associated mutations, and (iv) interpretation of the genotypic drug resistance / sensitivity profile.

Results: The detection of resistance-associated mutations revealed acceptable success rates of 81%-97% correct results in the EQA schemes 2016 to 2018. However, the success rates of 63%-97% for the interpretation of antiviral drug resistance against polymerase inhibitors were unsatisfactory for some samples of these three EQA schemes due to inconsistencies in the applied interpretation algorithms (mainly geno2pheno and HIV-GRADE) for certain mutations.

Conclusions: After harmonization of the algorithms geno2pheno and HIV-GRADE by an expert group of the Diagnostic Council of the German Association against Viral Diseases e.V. and the Society for Virology e.V., the success rates for the interpretation of the mutation M204V increased from 63% to 88%.

* Plasmids originated from the NRZ for HBV und HDV, Giessen, the group of Prof. Protzer, Muenchen, and the group of Prof. Nassal, Freiburg.
[P130] FIRST EVALUATION OF ALINITY M HIV, HBV AND HCV QUANTITATIVE ASSAYS IN CLINICAL ROUTINE

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Aim: "Viral load (VL) testing is the gold standard for therapy monitoring. Alinity m HIV, HBV and HCV assays on the fully automated random, continuous access Alinity m system* were approved recently. We present the first comparison of VL results measured with Alinity m and m2000sp/rt RealTime assays in clinical routine.”

Method: “Alinity m assays are designed with redundant detection and improved dynamic range in comparison to m2000sp/rt RealTime assays. In this retrospective evaluation, remnant samples (storage -80°C) with valid test results from 106 HIV, 101 HBV and 105 HCV EDTA plasma specimens comprising most prevalent subtypes/genotypes were retested with the Alinity m system.”

Results: “Viral load results were stratified according to the following categories: >LOQ (limit of quantification), quantification, <LOQ and “not detected”. The concordance between the HIV, HBV and HCV assays was 89.6%, 85.1% and 96.2%, respectively. Most of the discrepancies for HIV were observed with VLs <LOQ and “not detected” due to differences in LOQ between tests. Bland-Altman analysis demonstrated an overall mean bias (Alinity m/RealTime) for HIV, HBV and HCV of 0.09 log cps/ml, 0.05 log IU/ml and 0.27 log IU/ml, respectively. The correlation coefficient. R² ranged 0.96 - 0.99.”

Conclusions: “Alinity m showed good correlation and agreement with RealTime over the linear range for HIV, HBV and HCV. A high degree of sensitivity could also be confirmed for low viremic samples. Based on our first results a switch from m2000sp/rt to Alinity m appears to be safe in clinical routine.”

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SELECTION OF SPERMATOZOA FOR INTRACYTOPLASMATIC SPERM INJECTION IN CHRONICALLY HBV-INFECTED MEN

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Aim: HBV incorporates into spermatozoa reducing sperm concentration and motility, possibly presenting the need for intracytoplasmatic sperm injection (ICSI). HBV-incorporation could also cause paternofetal transmission during fertilisation. These safety concerns create reluctance to perform ICSI in chronically HBV-infected men. Assuming that spermatozoa with the highest motility are least HBV-incorporated, this study investigates an ICSI preparation technique to select non-infected spermatozoa, by isolating the most motile ones.

Method: Semen and blood samples were collected from 5 patients with chronic hepatitis B. Semen samples were analysed, processed by gradient centrifugation and incubated in a specific trajectory of gamete medium to separate spermatozoa into non-motile, motile non-progressive and motile progressive sample fractions. DNA was manually extracted (QIAamp DNA- Investigator-Kit) and HBV DNA loads were determined per fraction.

Results: Participants (averagely aged 31) were HBsAg+(5/5), anti-HBc+(5/5) and HBV DNA+(2/5). They were treated (3/5) with entecavir (1/5) or tenofovir(2/5) and had normospermia(5/5). CRP was detected in all sample fractions, proving successful DNA-extraction. In contrast, HBV DNA was not detected in any sample fraction, except for the motile, non-progressive fraction of 1 patient (HBeAg+, HBV DNA+).

Conclusions: Progressive fractions showed no detectable HBV DNA, although DNA-extraction proved successful, suggesting this technique a successful strategy to select non-infected spermatozoa for ICSI. Almost all fractions were HBV DNA negative, suggesting that well-treated patients with controlled disease have very low or no HBV DNA-contaminated semen samples. This study encourages re-evaluation of guidelines, restricting HBV-infected men from ICSI.
Hepatitis C Virus Subtyping in a Clinical Setting with Next Generation Sequencing Provides Additional Information Now and in the Future

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Aim: The increased accessibility to sequencing has encouraged the exploration of virus discovery and precise typing methods. Several studies have discovered that previous methods may misclassify subtypes and not recognize recombinant strains or new subtypes. In our clinical setting we wanted to use Next Generation Sequencing (NGS) to obtain near complete genomes for hepatitis C virus (HCV) for subtyping, resistance, and transmission determination.

Method: Samples were initially amplicon sequenced and now RNA-sequenced, since the latter methodology does not require a continuous update of primers or probes to include newly discovered subtypes.

Results: We have sequenced >400 HCV patient samples and obtained near complete genomes. These represent an overview of our regional everyday patient cohort combined with patients from study cohorts and allowed identification of the dominant clades in our region within genotype 1 and 3. Sequencing of routine samples have identified 3 recombinant viruses within genotype 2 and a genotype 6 genome with a currently unknown subtype. The near complete genomes made all currently relevant direct-acting antiviral agents (DAAs) targets accessible for investigation of resistance-associated substitutions (RASs) prior to treatment or after treatment failure. Future targets are most likely also included allowing subsequent analysis.

Conclusions: The availability of near complete genomes in our database during subtyping make additional analysis feasible without further lab work, when requested by the clinicians. Previous requests have been regarding potential RAS prior to retreatment after a failure, to determine if a treatment failed reinfection occurred and to verify potential transmission between patients.
CAN WE MINIMIZE SIGNAL CROSS-BLEED BETWEEN SAMPLES SEQUENCED ON AN ILLUMINA INSTRUMENT?

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Aim: The use of next-generation sequencers is cost-effective only when multiple samples are sequenced in parallel, being distinguished by dual indexing. However, signal from samples with high virus loads often cross-bleeds into positions sharing their row or column index. Here we present results from testing a protocol for minimizing signal cross-bleed using post hoc bioinformatic steps.

Method: The test data originated from a 96-well experiment with 83 blood samples from healthy children sequenced along with 8 different strong positive viral controls, no-template controls, and empty index combinations. The virome enrichment* was used. Two successive filtering steps were applied, eliminating reads (a) with any mismatches in indices, (b) with suboptimal index quality (mean index quality score < 32 or any base < 31). Data were processed using bcl2fastq, custom index-filtering scripts, and the VIPIE pipeline.

Results: Real samples had a median (IQR) of 194000 (103000-331000) raw reads, whereas non-existent and negative control index combinations were assigned 503 raw reads (179-680). Zero tolerance of index mismatches improved the data only marginally, but the subsequent elimination of reads with low-quality indices eliminated 86% (83%-91%) reads falsely assigned to control positions, whereas real samples were deprived only of 20% (17%-25%) reads. Positive controls and PCR-verified true enterovirus positivity remained unchanged upon this strict filtering, whereas numerous spurious low-positive findings disappeared (e.g. findings of noroviruses and sapoviruses viruses in blood).

Conclusions: The additional steps of index quality filtering profoundly decrease signal cross-bleed, although it cannot be fully eliminated by the sole use of bioinformatics.

* VirCapSeq-VERT
ELECTROSTATIC WIPES AS A SIMPLE AND RELIABLE METHOD FOR INFLUENZA VIRUS AIRBORNE DETECTION

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Aim: Airborne transmission of Influenza virus contributes to nosocomial infections but remains difficult to routinely monitor in healthcare settings. Here, we evaluated the performance of commercial electrostatic wipes (CEW) as simple, cheap and reliable supports for the detection of airborne viruses.

Method: We first impregnated 4 types of CEW with 5.9 log₁₀ copies/ml suspensions of a non-enveloped (adenovirus, ADV) or an enveloped (cytomegalovirus, CMV) virus to determine their analytical performances as supports for viral nucleic acid detection. Experiments were performed in quadruplicate. Then, a pilot study was conducted in rooms of Influenza-infected adult patients during the acute phase of the infection.

Results: Coefficients of variation (CV) between measurements was 4.8% for CMV (SD = 0.21) and 6.8% for ADV (SD = 0.29) showing a good repeatability of our protocol. Difference between viral DNA concentrations of the initial suspensions and the wipe washing fluids was 1.39 and 1.65 log₁₀ copies/ml for CMV and ADV, respectively, showing a high yield of our protocol. After impregnation with a suspension of Influenza virus (Ct value = 23.6) at room temperature, viral RNA was stably detected on CEW until 96h post-impregnation (ΔCq<1 ; SD = 0.24). After depositing CEW for 48h in 32 rooms of Influenza-infected patients, Influenza RNA was detectable in 75% (n = 24) of CEW, suggesting that our method accurately detects airborne virus in hospital settings.

Conclusions: CEW is a reliable, simple and inexpensive tool for the routine surveillance of the air contamination with Influenza virus in hospital settings.
P135 COMPARISON OF TWO COMMERCIAL QUANTITATIVE CYTOMEGALOVIRUS (CMV) PCR TESTS

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Aim: The aim of the study was to compare the results of two commercial CMV PCR tests which were calibrated with WHO International Standard.

Method: CMV DNA was investigated in 244 plasma samples by Artus CMV QS-RGQ (ACQR) Test* and Cobas Taqman CMV Test (CTMC), simultaneously after DNA extraction by QIASymphony* and Cobas Ampliprep (CAP)**, respectively.

Results: 174 samples were positive, 52 samples were negative, and a strong agreement was found between qualitative results of the tests (k=0.80, p<0.001). Sensitivity, specificity, positive predictive and negative predictive value of ACQR Test were; 92.6%, 92.9%, 97.8% and 78.8%, respectively, according to CAP/CTMC test. A maximum difference of 0.45 log10 was observed between the corresponding viral load values according to ROC analysis (Table 1). Bland-Altman analysis of quantitative results within dynamic ranges of the tests yielded a mean difference of −0.22 log10 for copy/mL and −0.47 log10 for IU/mL results to show that the ACQR measurements were higher than the CAP/CTMC test. According to linear regression analysis of quantitative results, a high correlation was found for both copy/mL and IU/mL results (r=0.94, p<0.001). Nineteen (14.5%) and 58(44.3%) samples had a measurement difference more than 0.5 log10 copy/mL and 0.5 log10 IU/mL. In 2 and 6 samples there was more than 1 log10 copy/mL and 1 log10 IU/mL difference between the tests.

Conclusions: In conclusion; qualitative results of both tests are well correlated. For quantitative evaluation; viral levels as copy/mL for both tests are more compatible than as IU/mL.

*Qiagen
**Roche Diagnostics
OPTIMIZED TRANSFER OF REAL-TIME RT-PCR ASSAYS FROM A 96- TO 384-WELL PLATE FORMAT ON THE SOLUTION SYSTEM* 

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Aim: We employ laboratory developed tests (LDT) against a multitude of targets on the Roche FLOW Solution System. To facilitate syndromic testing in a high-throughput setting, we transferred LDTs from a 96-well to 384-well plate format on LC480II. Here, we present an optimization and validation strategy used in transferring three assays, two multiplex assays** and one singleplex assay*** to a new plate format.

Method: In both well formats reaction volume was 20 µL, however, in the 384-well format input sample volume was reduced to 5 µL from 8 µL in the original 96-well format to allow syndromic testing from a single eluate. All primers and probes were re-titrated to establish optimal performance conditions in the new 384-well format. Assay performance was verified by comparing the Limit of Detection (LOD) using serial 4-fold dilutions of control material in both well formats. Additionally, external quality panels were run in parallel for each assay in both well formats.

Results: Titration of primers and probes in the 384-well format was essential for optimal performance of all assays. LOD increased comparable to the reduced input sample volume in the 384-well format, except for the Sapovirus1245 assay for which LOD increased 4-8 fold. In general, Cₚ-values increased, and endpoint fluorescence levels obtained lowered in the 384-well format compared to the 96-well format despite prior optimization.

Conclusions: Existing and well characterized PCR assays require re-optimization for successful transfer to 384-well format and re-validation before implementation in diagnostic routine.

* Roche FLOW
** Adenovirus40/41 + RotaA and Sapovirus1245 + NorovirusGI
*** NorovirusGII
VIRUS DISCOVERY USING METAGENOMICS IN A CLINICAL SETTING; THE REDISCOVERY OF MIDDLE EAST RESPIRATORY SYNDROME AND SEVERE ACUTE RESPIRATORY SYNDROME

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Aim: Metagenomic shotgun sequencing has the potential to detect rare but also previously undiscovered pathogens. In this study we learn how unknown low abundant virus reads would be handled by our bioinformatic metagenomics pipeline.

Method: Two clinical respiratory samples were sequenced utilizing shotgun metagenomics. Over 10 million reads per patient sample were in silico spiked with 1,000-20,000 MERS and SARS corona virus reads. To mimic the setting of virus discovery, we classified the spiked dataset using Centrifuge with a reference index of Refseq viruses including only corona viruses prior to 2003, the year of the discovery of SARS. After extraction of unassigned reads, we assembled and analysed genome contigs.

Results: After classification, one million reads were unassigned and 10-200 of the 1,000 and 20,000 spiked reads were assigned to corona viruses from before 2003. From the extracted unassigned reads ~60,000 contigs were assembled of which 100 contigs were well-covered and >500 nucleotides long. In total <25 contigs blasted to the MERS or SARS genome with an identity of 99-100%.

Conclusions: We traced back our spiked-in MERS and SARS reads with great accuracy. Parts of the larger built viral contigs partly resembled Coronaviridae species prior to 2003. In the future, such a finding could indeed indicate the presence of a novel virus in a clinical sample. Our results provided insight for further developing a sensitive virus discovery workflow. The identification of both known and novel pathogens directly in clinical patient samples by means of a catch-all metagenomic assay will be paradigmatic.

Schematic overview of rediscovering SARS in clinical patient spiked with 1000 SARS reads
VIROME STUDIES BY RANDOM FRAGMENTS SEQUENCING OR BY VIRUS PROBE-BASED APPROACH: WHICH IS BETTER?

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Aim: We aimed to compare two different approaches for sample preparation for study of virome in terms of sensitivity and specificity to known viruses as well as their variants.

Method: Starting material was stool suspension from 178 healthy diabetic children from four African – Asian countries, median age 11.5 years [7.8 – 14.0]. After total viral nucleic acid isolation random fragments were prepared. In the first approach all pre-amplified random fragments were sequenced and libraries were prepared by Nextera XT, Illumina. In second approach random fragments were enriched for viruses infecting vertebrates by using VirCapSeq-VERT probes implemented in NimbleGen library preparation design, Roche. Sequencing was done on MiSeq instrument, Illumina with 2x250bp sequencing kit. Reads were then assembled into contigs and the closest references were identified by using NCBI database.

Results: Of the total number of sequences viruses were identified in 5.67% by probe-enriched samples compared to 0.376% by all random fragments sequencing approach (3.86% including bacteriophages and plant viruses). Probe-enriched samples approach identified 16 viruses infecting humans and 4 infecting other vertebrates. All fragments sequencing approach identified 6 viruses infecting humans, 1 virus infecting other vertebrates, 5 bacteriophages and 2 plant viruses. All samples positive for a human virus by all fragments approach were confirmed by probe-based approach, which identified another 24 samples positive for a human virus.

Conclusions: Probe-based approach proved to be more sensitive for known viruses that infect vertebrates while all fragments sequencing approach has a potential to sequence also other kinds of viruses, such as bacteriophages.
Aim: The objective of this study was to characterize performance of the CMV Test* across key performance metrics the sytsems**.

Method: A wide range of analytical studies including characterizing sensitivity, linearity & quantitation limits, cross-reactivity, inclusivity, precision, effect of interfering substances, and method correlation were performed. Analytical sensitivity was determined using the 1st WHO International Standard for CMV and the quantitation limits (LLoQ/ULoQ) were determined using the TAE ≤1.0 criterion. Secondary standards traceable to the 1st WHO CMV international standard were used for the rest of the analytical testing. The Method Correlation study was performed using remnant clinical specimens.

Results: Evaluation of analytical sensitivity of the CMV Test* gave an LoD and LLoQ of 20 IU/mL. The CMV Test* demonstrated excellent linearity across clinically relevant >6 log dynamic range with a slope of 0.99 as well as quantitative precision across 3 systems over 12 days, and quantitative equivalence across multiple reagent lots. Turnaround time (TAT) for the CMV Test* was ~60 min. No cross-reactivity was detected against any of the 35 non-target pathogens tested and there was no interference observed against the 33 endogenous or exogenous agents tested. A method correlation study conducted between the CMV Test* and the reference tests showed excellent linear correlation and a bias of 0.24 log_{10} IU/mL.

Conclusions: The CMV Test* demonstrated excellent performance and will be a crucial tool for use in viral load monitoring in the critical organ transplant patient population.

*NeuMoDx
** NeuMoDx 288 and NeuMoDx 96
Aim: The objective of this study was to characterize performance of the EBV Test* across key performance metrics on the systems**.

Method: Analytical studies were performed using plasma and whole blood matrices to characterize various analytical parameter such as - sensitivity, cross-reactivity, inclusivity, interfering substances, cross-contamination, specimen stability, and turnaround time (TAT).

Results: The EBV Test* demonstrated a limit of detection (LoD) of 19 IU/mL and a LLoQ of 20.2 IU/mL in plasma and an LoD of 80 IU/mL with an LLoQ of 100 IU/mL in whole blood. The EBV Test* showed excellent linearity across a 7-log dynamic range ($R^2 > 0.98$) with ULoQ of 7 Log10 IU/mL. Excellent quantitative precision across 3 systems over 12 days (maximum deviation of 0.15 Log10 IU/mL), as well as quantitative equivalency across multiple reagent lots was demonstrated. The integrated results interpretation module provided extremely accurate results with a time to first results of only ~60 minutes. No cross-reactivity was observed against any of the 35 non-target pathogens tested, and the test performed efficaciously in the presence of endogenous and exogenous interfering moieties. Finally, equivalent performance was demonstrated across plasma (fresh and frozen) and fresh whole blood specimens.

Conclusions: The EBV Test* demonstrated excellent performance and is well suited for implementing viral load monitoring using both plasma and whole blood specimens.

* NeuMoDx
** NeuMoDx 96 and 288
Aim: Universal transport media (UTM) is well-known to simplify collection, transport and maintenance of viruses, chlamydia, mycoplasma and ureaplasma specimens. In order to establish the performance of UTM (COPAN Italia) according to “Quality Control of Microbiological Transport Systems” (CLSI M40-A2) an investigation of viability of ten clinically important pathogenic strains, at 4°C and room temperature (RT 20-24°C) over 48 hours of incubation was conducted.

Method: Viral culture stocks were obtained from the American Type Culture Collection (ATCC). The following pathogens were considered: Herpes simplex virus type1 (ATCC VR-539); Herpes simplex virus type2 (ATCC VR-734); Respiratory Syncytial Virus (ATCC VR-1580); Coxsackie B1 (ATCC VR-28); Influenza A virus (ATCC VR-1679); Cytomegalovirus (ATCC VR-977); Varicella Zoster Virus (ATCC VR-1367); Mycoplasma pneumoniae (ATCC 15331); Chlamydia trachomatis (ATCC VR-880) and Chlamydia pneumoniae (ATCC VR-1360). UTM medium was inoculated with a known concentration of each strain (enough to infect 40-50% of cell monolayer or PPLO selective agar). At time zero and after 48hr, to establish the viability of each strain, an aliquot of UTM medium was used to infect 24 wells cell culture plates or PPLO selective agar. After plate incubation under the appropriate conditions, infected cells (using immunofluorescence) or CFUs (colony forming units) were counted.

Results: After 48h from inoculum, at both temperatures, we observed for each strain, less than 50% reduction in fluorescing infected cells (or CFUs), with respect to time zero, according to CLSI M40-A2.

Conclusions: Based on data obtained, Copan UTM is able to guarantee its compliance to the M40-A2 guideline.
Aim: “Our objective is to assess the prevalence and distribution of persistent DNA viruses in soft tissues. More specifically, we aim to investigate the viral loads as well as the within-host diversities of the viral DNA sequences.”

Method: “We collected post-mortem samples from brain, skin, colon, liver, lung, heart, kidney, hair follicles and blood of 30 recently deceased individuals. The DNA of viruses belonging to the families Parvoviridae, Anelloviridae, Polyomaviridae and Herpesviridae were screened by i) quantitative PCRs and ii) targeted enrichment via in-solution capture with virus-specific biotinylated RNA probes followed by next generation sequencing.”

Results: “Preliminary results show a prevalence of 87% for parvovirus B19, with hair samples being solely negative for this virus. Polyomaviruses MCV and JCV were found sporadically in the tissues studied, altogether in 14/30 and 7/30 individuals, respectively. EBV was detected in 22/30 individuals, most frequently in the lung. TTV was found in all the tissue types studied with highest prevalence in blood and liver of 23/30 individuals.”

Conclusions: “Many human DNA viruses are known to establish tissue persistence. However, the current knowledge is based mainly on the detection of viruses using conventional assays in particular tissue types, providing thus an incomplete picture of viral distribution among the organs of a given individual. The data show a virus-unique distribution and distinct loads in the different organs of an individual. A systematic study on the divergence of viral DNAs within the human provides an important baseline in the study of the human virome.”
[P143] EVALUATION OF THE NEW HSV1&2 VZV KIT* FOR HSV1, HSV2 AND VZV LOAD MEASUREMENT

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Aim: Quantitative PCR (qPCR) is essential for the diagnosis of HSV and VZV infections. The new HSV1&2 VZV kit* (69-014B under development**) for measurement of HSV1, HSV2 and VZV loads uses 30% less eluate volume than the previous kit (HSV1 HSV2 VZV*, 69-004B) due to simultaneous detection of HSV1, HSV2 and internal control. In this study, the qualitative and quantitative performances of this new assay were evaluated in comparison to the 69-004B.

Method: 654 samples for HSV1, 791 for HSV2 and 361 for VZV (including WB, plasma, CSF, BAL, anogenital, mucocutaneous and throat swabs) were tested for qualitative analysis, and viral loads measured in all matrices except swabs. Monitoring (range: 2-8 samples) was possible for 21 patients.

Results: The qualitative analysis showed a high agreement between kits: 97.2% for HSV1, 95.8% for HSV2 and 98.6% for VZV. The quantitative analysis for each target showed a high correlation between kits (R ranging from 0.9511 to 0.9956 according to virus and sample type). Mean bias absolute values for the new kit varied from 0.01 log10 copies/ml for HSV-2 and VZV to 0.78 log10 copies/ml for HSV-1. The new kit showed higher specificity for HSV-1 and higher sensitivity for HSV-2. Longitudinal viral monitoring showed similar kinetics for both kits.

Conclusions: The HSV1&2 VZV kit* showed similar performance for VZV and improved performance for HSV1 and HSV2 compared to the previous kit and uses less eluate volume, which makes it especially suited to routine practice.

* R-GENE®, **ARGENE®, BIOMERIEUX
SUITABILITY OF SALIVA SAMPLE AND LYSIS PROTOCOL FOR CMV DNA DETECTION: A PRELIMINARY STUDY

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Aim: CMV DNA detection is routinely carried out by qPCR on blood and urine; saliva has been proposed for CMV neonatal screening programs. This study compares CMV DNA detection in saliva samples versus blood or urine. A rapid lysis protocol for saliva was evaluated.

Methods: 10 negative subjects (Group I) and 20 patients (IgM-IgG positive, Group II) were selected for saliva collection using a nylon FLOQSwab® stored in MSwab medium. For Group II, blood and urine samples were also collected. For saliva, blood and urine CMV DNA was obtained using an automatic extraction system. Nucleic acids for saliva specimens of both groups were extracted using a direct heat lysis protocol. qPCR assay was finally carried out for all samples.

Results: Group I was confirmed CMV negative with both extraction methods. In Group II, 12 samples were CMV positive in saliva, urine and/or blood; 4 samples were negative for all matrices. One sample resulted positive only in saliva, while three were negative in saliva and positive in urine or blood. No significant differences between lysis and extraction methods in CMV detection was observed in saliva samples.

Conclusions: Saliva is suitable to detect CMV DNA comparing to blood and urine, particularly in population studies or neonatal screening. Different results were observed among saliva, blood, and urine possible due to individual different times of infection. MSwab direct-heat-lysis can be an optimal method for screening newborns, followed by confirmation of viral DNA positivity on blood and/or urine.

1MSwab® (Copan)
2MagnaPure (Roche Diagnostics)
3REALQUALITY RQ-CMV (AB ANALITICA)
[P145] ACCURACY OF IGM POSITIVE SEROLOGICAL PROFILES DURING CYTOMEGALOVIRUS, EPSTEIN-BARR VIRUS OR PARVOVIRUS B19 ACUTE INFECTIONS

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Aim: Serological IgM cross reactivity between cytomegalovirus (CMV), Epstein-Barr virus (EBV) and parvovirus-B19 (B19) impairs acute infection diagnosis. The objectives of this study were to analyze the incidence of IgM false reactivity and its clinical consequence.

Method: Among 2538 IgM-tested samples over a one-year period, 309 were positive for at least one CMV, EBV or B19 IgM. Samples tested for at least 2 IgM (n=165) were identified and IgM detection for the 3 viruses was completed when required. Overall, 150 complete IgM profiles were available for analysis. Retained final diagnosis for each infection was based on virus molecular testing and/or clinician conclusions.

Results: IgM reactivity was found for 1, 2 and 3 viruses for 98 (66%), 34 (22%) and 18 (12%) samples, respectively. One or more IgM false reactivity have been confirmed for 115 patients (77%). Among IgM positive samples for at least two viruses, coinfections have been proved in 19% (10/52). Results solely focused on IgM+ but IgG-negative profiles are summarized in the table.

<table>
<thead>
<tr>
<th>IgM+/IgG-</th>
<th>B19</th>
<th>CMV</th>
<th>EBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Median age-[range]</td>
<td>4-[0;34]</td>
<td>9-[2;76]</td>
<td>8-[1;34]</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Final diagnosis with proven infections</th>
</tr>
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<tbody>
<tr>
<td>B19</td>
</tr>
<tr>
<td>CMV</td>
</tr>
<tr>
<td>EBV</td>
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<tr>
<td>IgM false reactivity</td>
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Conclusions: This study highlights the risk of false positive IgM, reaching up to 92% for acute CMV infection diagnosis. A more reliable approach may imply IgM determination for the 3 viruses, manufacturer's threshold refinement and molecular testing.
Aim: We aimed to examine the sensitivity of several commercial point-of-care diagnostic devices compared with a laboratory in-house test, and to examine if there is a mismatch between the primers used in these commercial point-of-care diagnostic tests and the strain used for the WHO universal standard.

Method: We compared the performance of the systems* with our laboratory in-house test for sensitivity. To do this, we used serial dilutions of two strains of HSV1 and HSV2 between a concentration of 6.39 and 1.68 log_{10} copies/mL, provided by QCMD (Quality Control for Molecular Diagnostics). We compared the limit of detection of point-of-care diagnostic tests with the viral load found in meningitis and encephalitis patients, in order to find the necessary sensitivity for diagnostic purposes.

Results: Preliminary results show that none of the rapid tests we examined can compete with the sensitivity of an in-house assay. Differences between rapid tests exist between HSV1 and HSV2, as well as between strains. Based on data from patients, we argue that for diagnostic use it is necessary to at least be able to detect samples of 3.0 log_{10} copies/mL and higher.

Conclusions: We conclude that the sensitivity of most point-of-care diagnostic tests is insufficient for clinical use, due to a high risk of false negatives for either HSV1 or HSV2. However, their improved turn-around-time and ease of use could potentially benefit molecular diagnostic laboratories.

*FilmArray Torch^1, InGenius^2, and the Argene^1

1^ bioMérieux
2^ EliTechGroup
CONTINUING HARMONIZATION OF HCV RNA MEASUREMENTS BY NAT

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Aim: Viral load measurements using nucleic acid amplification techniques (NAT) are critical for the prevention and management of hepatitis C virus (HCV) infections. The WHO International Standard for HCV RNA defines the detection and quantification specifications for HCV RNA in International Units. Here we describe the collaborative study to establish a replacement HCV International Standard and evaluate cell cultured HCV (HCVcc) as a potential source material for future HCV references.

Method: Two candidate replacement standards, each comprising lyophilized HCV plasma were prepared and evaluated in a multicentre study involving 19 laboratories from 12 countries worldwide. The candidates were evaluated alongside samples of inactivated HCVcc and HCV-positive plasma donations. Study participants tested dilutions of each sample prepared in negative plasma using their routine HCV NAT-based assay.

Results: Most participants used commercial quantitative HCV NAT assays based on real-time PCR technology. The standard deviation of mean estimates (inter-laboratory variation) from quantitative assays for each sample ranged from 0.11 to 0.35 log10 IU/mL. The overall mean potency estimates for candidates 1 and 2, were 5.35 and 5.34 log10 IU/mL respectively, relative to the existing International Standard.

Conclusions: The results of the study indicate the suitability of both candidates as replacement 6th HCV WHO International Standards. This reference continues to be an important tool for harmonizing HCV RNA measurements. HCVcc could provide an alternative long-term source material for this reference.

We thank Dr Wakita (NIID, Japan) for providing the HCVcc used in the study. We gratefully acknowledge the contributions of the collaborative study participants.

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Aim: Encephalitis and meningitis are infectious diseases of the central nervous system (CNS) which can have severe sequelae even be fatal. Early diagnosis of the pathogens is important for proper treatment, which may prevent life-threatening clinical manifestations. In this study we evaluated the performance of *multiplex one-step Real-time RT-PCR assay for diagnosing 18 known causal pathogens including 6 bacteria and 12 viruses simultaneously, using the Seegene’s proprietary qPCR technology**.

Method: The analytical sensitivity of the *multiplex one-step Real-time RT-PCR assay was analysed using dilutions of well-defined reference strains. For the clinical validation experiments, cerebrospinal fluid (CSF) from patients with meningitis were analysed. The results were compared with in-house RealTime PCR tests and the results of bacterial culture experiments.

Results: In the validation study a high detection rate of 100% compared to culture and in-house RealTime PCR test were obtained. Neither cross reactivity, nor false-positive results were observed.

Conclusions: *Multiplex one-step Real-time RT-PCR assay showed a high sensitivity for the detection of the most common pathogens in clinical CSF samples. This kit is very useful for the identification of acute meningitis-causing pathogens.

* the Allplex™ Meningitis Panel Assays (Seegene Inc., Korea)
** MuDT™ technology
ENTEROVIRUS PREVALENCE AND RECOMBINATION IN A MALAWIAN COHORT

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Aim: Enteroviruses are among the most prevalent viruses infecting humans worldwide. We know that EVs can recombine; poliovirus (PV) for example can recombine with other EVs within subspecies C (EV-C). PV from life attenuated vaccines, widely used in sub-Saharan Africa, can regain virulence by recombining, and cause outbreaks of polio-like disease. The aim of our present research was to report the EV prevalence in a Malawian cohort, and to study EV-C recombination.

Method: We tested 749 fecal samples from Malawian children on the presence of EVs, and typed the positive samples using Viral Protein 1 (VP1) sequences. We constructed phylogenetic trees of a dataset containing our own EV-C strains and EV-C strains from GenBank and scanned the full genomes for recombination breakpoints.

Results: Of all samples, 90% was EV positive. The phylogenetic trees of EV-C showed phylogenetic violation, and recombination breakpoints were identified in the non-structural parts of the genome. While recombination was possible between two subspecies of EV-C – subspecies B and C –, recombination in subspecies A was more restricted, and only happened within, but not between, three separate clusters.

Conclusions: The EV prevalence in our cohort was extremely high. We saw phylogenetic violation within EV-C, suggesting recombination. The recombination dynamics differed between the subspecies in species EV-C. This might be partially explained by prevalence and tropism of the individual serotypes.

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PARECHOVIRUS PREVALENCE AND TYPING IN A MALAWIAN COHORT

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Aim: Parechoviruses (PeVs) are highly prevalent viruses worldwide. However, little is known about the prevalence of these viruses in Africa. The aim of our present research was to study the prevalence of PeV in a Malawian cohort.

Method: We tested 749 fecal samples from Malawian children on presence of PeVs and sequenced the complete Viral Protein 1 (VP1) region of the positive samples. For typing, we used four different, widely used, methods: applying Basic Local Alignment Search Theorem (BLAST) to the VP1 sequences, constructing a Maximum Likelihood (ML) phylogenetic tree based on the VP1 sequences, constructing a Neighbor Joining (NJ) tree based on the VP1 sequences, and constructing a ML tree based on the VP3/VP1 junction region.

Results: We found a PeV prevalence of 57%. When typing the strains, we found that the results of the different typing methods showed several inconsistencies. While the NJ- and ML-tree on VP1 sequence identified 15 different genotypes, BLAST resulted in 14 different types. In the VP3/VP1 tree, two strains clustered with a different genotype compared to the VP1 trees.

Conclusions: We found a remarkably high PeV prevalence and diversity in our cohort. We saw that different typing methods, all frequently used for typing PeV strains, resulted in dissimilar typing for the same viral strains. This could lead, and possibly already has led, to inconsistent or incorrect typing of PeV strains. We are currently developing a typing framework that is more applicable for PeVs, and that will prevent typing inconsistencies in the future.
[P151] HUMAN HERPES VIRUS INFECTIONS IN BULGARIAN PATIENTS WITH MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME

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Aim: Infections of cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus-6 (HHV-6) are suspected as etiological agents for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Our aim was to determine frequency of EBV, CMV and HHV-6 active/persistent infection in bulgarian patients with ME/CFS.

Method: Blood samples from patients with ME/CFS (n=48) and healthy controls (n=40) were included in the study. DNA from plasma and peripheral blood mononuclear cells (PBMCs) was tested for CMV, EBV and HHV-6 by PCR. Statistical analysis was performed using Fisher exact test (p < 0.05 considered statistically significant).

Results: In ME/CFS plasma samples, EBV DNA was found in 16/48 (33.3%), CMV DNA – in 3/48 (6.3%) and HHV-6 DNA in 1/48 (2%) of samples. EBV DNA was detected in 2/40 (5%), CMV and HHV-6 DNA were not found in plasma samples of controls. The difference in frequency of EBV active infection in ME/CFS and control group was statistically significant (p = 0.0011). There was no statistically significant difference for CMV and HHV-6. The frequency of viral genome sequences detection in PBMCs of patients and controls was 81% vs 80% for EBV and HHV-6 and 75% vs 80% for CMV.

Conclusions: There is a significant difference in the frequency of EBV active infection in ME/CFS patients compared to controls, suggesting a possible role of EBV in ME/CFS pathogenesis. There are high but similar frequency rates of EBV, CMV and HHV-6 persistent infections among ME/CFS patients and healthy persons.
[P152] HUMAN PARECOVIRUS INFECTIONS IN SPAIN, 2011-2019

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Aim: Human parechoviruses (HPeV), as well as enteroviruses, have been recognized as important causes of severe illness among young children. We investigated the epidemiological and clinical characteristics of HPeV infections in Spain over an 8-year study period.

Method: Study included 152 HPeV-positive samples from 144 hospitalized patients identified between March 2011 and February 2019. HPeV genotyping was performed by RT-PCR and further sequencing. Phylogenetic analysis in VP1/VP3 region was also carried-out.

Results: The 152 HPeV-positive specimens were CSF (60%), blood (30%), throw swabs (7%) and stools (3%). HPeV3 was the prevalent type (127/152, 83.5%) followed by HPeV1, HPeV4 (7 each, 4.6%), HPeV5 (4, 2.6%) and HPeV6 (2, 1.3%). Five HPeV (3.2%) could not be typed. The mean age of the patients was 2.1 months (range, 1day-36 months). The highest incidence of HPeV infections was between April and July (58% of total detections), with another small peak in autumn (17%). Furthermore, HPeV-3 circulated more frequently every 2 years. HPeV infections were mainly associated with no-specific febrile syndrome (N=80, 55%), meningitis or meningoencephalitis (N=45, 31.2%) and sepsis-like disease (N=12, 8.3%). HPeV were also identified in respiratory diseases (N=4) and in 3 paralysis/ataxia cases.

Conclusions: This is the largest study about characterization of HPeV infections performed in Spain so far. Results supported that HPeV3 is the prevalent type associated with neurological pathologies and sepsis in young children, but other types can be also implicated. Biennial circulation of HPeV3 was confirmed. HPeV have to be included in the routine diagnosis of these syndromes.
[P153] PROSPECTIVE EVALUATION OF RAPID SYNDROMIC MOLECULAR PANEL IN PATIENTS WITH MENINGITIS AND ENCEPHALITIS

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Aim: In this study, we aimed to investigate the prevalence of bacterial, fungal and viral pathogens and evaluation of rapid syndromic panel in patients with meningitis and encephalitis.

Method: Cerebrospinal fluid (CSF) samples were collected from 314 patients with central nervous system infections. Among the patients, 165 (52.5%) were male and 149 (47.5%) were female. The age range of patients is between 36 days to 88 years (median: 34.5 years). All specimens were tested by FilmArray Meningitis/Encephalitis Panel*. CSF culture and identification were performed using conventional microbiological tests. Viral multiplex PCR kit (FTD-Neuro 9, Fast Track Diagnostic) was used to confirm all positive viral samples.

Results: Among the 314 tested specimens, 29 (9.2%) were positive. In the 29 positive specimens, 11 were Herpes Simplex virus (HSV1), nine were Streptococcus pneumonia, and nine were other pathogens (two HHV6, two VZV, two N. meningitidis, one Enterovirus, one S. agalactiae, one C.neoformans/gattii). 11 of the 16 virus-positive samples were confirmed by FTD test and clinically supported. Three samples positive for HSV1 by MEP were found negative by FTD test and clinically. Two samples with HHV-6 positive were confirmed by FTD test but these patients were not clinically supported. All patients with bacterial pathogens had purulent meningitis and CFS abnormalities. Time-to-diagnosis mean with MEP was found to be 3.8 hours.

Conclusions: The FilmArray M/E Panel enabled accurate and fast diagnosis of meningitis/encephalitis cases at the four hours of admission. Three HSV1 positive MEP results were considered false positive relative to the comparator method. HHV6 was evaluated as a bystander in two patients. Overall positivity rate was found 7.6% in these patients.

*MEP-BIOFIRE, Salt Lake City, UT
[P154] ECHOVIRUS 30 SURVEILLANCE. TWENTY-THREE YEARS OF EXPERIENCE.

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Aim: To assess the clinical and epidemiological characteristics of echoviruses 30 (E-30) infections detected during a 23 year-period.

Method: We retrospectively recorded the data of E-30-positive cases detected in a University Hospital throughout 1996-2018. Detection of E-30 was accomplished by cell culture and/or PCR. Genotyping was done by PCR and sequencing.

Results: E-30 was detected in 228 specimens (86 cerebrospinal fluids (CSF), 98 respiratory specimens, 40 feces and 4 others) from 169 patients. The male/female rate was 1.5, and the age ranged from 7 days to 71 years, 50% were under 3 years. Clinically, infection by E-30 was associated with central nervous system (CNS) involvement in 106 (63%) cases, febrile syndrome in 47 (28%), respiratory diseases in 12 (7%) and skin lesions in 4 (2%). In 72.8% of the patients, only one specimen was analyzed. In 37 patients with CNS involvement multiple specimens were analyzed. In those cases, E-30 detection efficiency was 73% in CSF, 80% in respiratory specimens and 100% in feces. E-30 was detected on almost all recorded years (Figure) showing increasing detections every two to three years. Cases were detected throughout all year with approximately half of them presented between May and July.

Conclusions: Most of the E-30 was detected from patients with CNS involvement (63%). Efficiency in the detection of E-30 in CSF from CNS involvement cases was only 73%. E-30 is considered endemic in our area since it circulates almost every year.
EMERGENCE OF ENTEROVIRUS A71 SUB-GENOTYPE C4 IN GERMANY, 2018

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Aim: “Enterovirus A71 (EV-A71) is currently separated in several sub-genotypes. In Europe, EV-A71 has been detected only sporadically in patients with neurological disorders, with C2 being the predominant sub-genotype during the last decade. A recombinant C1-like sub-genotype emerged in 2015 in Germany and has been the cause of a large brainstem encephalitis outbreak in Spain in 2016. In South-East-Asia, EV-A71 sub-genotype C4 has been primarily associated with severe courses of hand, foot, and mouth disease (HFMD) in children. C4 viruses were first detected in Germany in 2013 within the national enterovirus surveillance (EVSurv).”

Method: “Surveillance of enteroviruses is based on stool or CSF samples collected from patients with suspected viral meningitis/encephalitis (M/E) or acute flaccid paralysis. In addition to EVSurv, samples from patients with HFMD are sent for typing to investigate outbreaks in nursery schools.”

Results: “In 2018, 1,959 samples were tested within the EVSurv with 416 (21.2%) being positive for enteroviruses and 40 being identified as EV-A71. By VP1 sequencing, 21x EV-A71 C1-like and 8x EV-A71 C4 were subtyped. 11 strains were not further sequenced. In addition, within stool samples collected from HFMD patients, 4 were identified as EV-A71 C4. No severe courses were reported. Sequence analysis showed high nucleotide similarities to EV-A71 C4 strains detected in China 2016/2017. No amino acid differences in the VP1 region were identified between M/E and HFMD cases.”

Conclusions: “Continued surveillance of hospitalized patients and outpatients with subsequent sequence analysis is needed to investigate enterovirus circulation.”
A SEVERE CASE OF AFP: THINK BEYOND EV-D68 AND EV-A71

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Aim: In the post-polio era, enterovirus (EV)-D68 and EV-A71 are the most notorious viruses to cause acute flaccid paralysis (AFP). Here we present a case of AFP with severe clinical outcome caused by another EV.

A 5 month-old boy was transferred to our hospital and admitted to the pediatric ICU with a clinical presentation of AFP. This was confirmed with MRI-scans which showed extensive myelitis. Guillain–Barré-syndrome was excluded and AFP induced by an (entero)viral infection was suspected. Six weeks later there was still no amelioration of his clinical condition and he is still mechanically ventilated via tracheostomy and sedated.

Method: In the context of AFP investigation a nasopharyngeal aspirate, a cerebrospinal fluid (CSF) and two stool samples were collected and tested for pathogens. These samples were also sent to the National Reference Centre (NRC) for EV for sequencing.

Results: In our lab rhinovirus (RV) and human bocavirus were detected in the respiratory specimen. NRC confirmed RV-A in the respiratory sample and detected a coxsackievirus A9 (CV-A9) and bocavirus in the stool sample by next-generation-sequencing. There was no pathogen detected in the CSF.

As CV-A9 is sporadically described as a neurotropic virulent EV, we rather think that this isolate – and not RV-A or bocavirus – was the causative agent. To gather more evidence of CV-A9 causality, additional tests on CSF, including intrathecal EV-antibodies, are planned.

Conclusions: This case shows the importance of analysing multiple samples, and the search for other EV’s than EV-D68 or AV-A71 as the cause of severe AFP.
EVALUATION OF A COMMERCIAL MOLECULAR ASSAY FOR THE DIAGNOSIS OF VIRAL ENCEPHALITIS AND CORRELATION WITH CEREBROSPINAL FLUID CELL COUNTS IN PEDIATRIC HOSPITALIZED PATIENTS.

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Aim: Recently, the presence of neurological viruses in cerebrospinal (CSF) fluid with pleocytosis (cells <5/mm³) in patients <3 months of age was reported. The present study investigated the performances of a commercial multiplex molecular assays (1) together with the CSF profile, including cells count and protein level.

Method: A total of 123 of CSF collected from pediatric patients (median age 5 months, range 1 day–16 years) for virological diagnosis were retrospectively tested using commercial molecular assays (1) analyzing the following targets: herpes virus type 1 and 2 and varicella-zoster virus, enterovirus, parechovirus and adenovirus. Protein level and cell counts were retrieved for all CSF.

Results: A total of 14/123 (11.4%) CSF were detected as positive. Of them, 9/14 were positive for enterovirus, 3/14 for parechovirus, 1/14 for varicella and 1/14 for herpes type 2. Forty-four out of 123 (43.9%) CSF had ≥5 cells/mm³, while 69/123 (56.1%) had <5 cells/mm³. Interestingly, half of virus-positive (7/14; 50.0%) CSF belonged to the group with <5 cells/mm³ including 4 enterovirus, 3 parechovirus and 1 varicella infections. Seventy-six out of 123 (61.8%) CSF had protein levels<50 mg/dl, while 47/123 (38.2%) had ≥50 mg/dl. Half of virus-positive CSF had protein levels<50 mg/dl. Among enterovirus-positive group, patient with <5 cells/mm³ were younger than patients with ≥5 cells/mm³ (25 days vs 55 days; p=0.03).

Conclusions: Molecular diagnosis of viral encephalitis should be performed also in CSF without pleocytosis especially in patients <3 months of age.

FUNCTIONAL ANALYSIS OF NUCLEOCAPSID PROTEIN BINDING HOST PROTEINS IDENTIFIED BY PROTEIN MICROARRAY IN SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS INFECTION

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Aim: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging high-fatality infectious disease caused by a SFTS phlebovirus (SFTSV) in China, Japan, and Korea. Because there are currently no licensed vaccine and effective therapeutics for the treatment of SFTS, we investigated host-virus interaction be able to contribute to the development of antiviral strategies.

Method: SFTSV nucleocapsid protein (NP) is involved in regulation of viral life cycles including RNA encapsidation, transcription, and replication. However, study on host targets to regulate the function of NP in SFTSV infection is limited. In this study, we identified host cell proteins as NP targets using protein chip microarray technology. To investigate the interaction between NP and cellular targets in SFTSV infection, we generated shRNA-mediated knockdown cells using lentiviral systems.

Results: We identified 376 host cell proteins as NP targets using protein chip microarray technology. Analysis for their gene ontology biological process, molecular function, and cellular component showed significant enrichment for categories related to metabolic and protein modification process, response to stress, and cell death. Among them, 6 host proteins were interacted with NP by co-immunoprecipitation (Co-IP) in co-transfected cells. Further studies for analysis of the detail function of host proteins are ongoing.

Conclusions: Our results provided useful information for the development of efficient antivirals for SFTSV.

* This study was supported by intramural funds (2017-NI53003-02) from the National Institute of Health, Korea CDC

Key words: SFTSV, Protein chip microarray, Nucleocapsid protein
Aim: Human parechoviruses (HPeVs) are widespread pathogens belonging to the Picornaviridae family and currently divided into 19 genotypes. HPeV infections can be associated with severe clinical manifestations, such as sepsis-like illness, particularly in youngest children. The epidemiological and molecular characteristics of HPeV infections observed in children <6 months hospitalized with symptoms of sepsis-like illness were investigated.

Method: From January 1st, 2015, to December 31st, 2018, clinical samples (cerebrospinal fluid samples and/or blood samples) were collected for diagnosis of HPeV infection from 193 patients (median age: 21 days, range: 1 day - 6 months) hospitalized with symptoms of sepsis-like illness, in two hospitals of Northern Italy. HPeV-RNA was detected by real-time RT-PCR (target 5'UTR) and a portion of HPeV VP3/VP1 junction (nt. 2159–2458) was sequenced for typing and molecular characterization.

Results: 14% (27/193) of patients with symptoms of sepsis-like illness tested HPeV-positive. 26/27 (96.3%) HPeV-cases were <3 months and 20/27 (74.1%) <1 month. HPeV-positive cases were detected throughout the study period, mainly (12/27; 44.4%) during the summertime (June-August). 17/27 (63%) HPeV-positive samples were molecularly characterized: 16 resulted HPeV-3 and 1 HPeV-5.

Conclusions: HPeV infection was identified in 14% of children <6 months with symptoms of sepsis-like illness. Almost all HPeV infections were detected in children <3 months and mainly during the summertime; almost all molecularly characterized HPeV belonged to type 3. Including HPeV molecular detection in routine diagnostic tests would allow estimating the burden of HPeV infection and improving clinical management of pediatric patients.
DETECTION OF HUMAN HERPES VIRUS 6 VARIANTS HHV-6A & HHV-6B IN IRELAND 2018-2019

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Aim: "Following the discovery of Human Herpes Virus 6 (HHV-6) in 1986, two distinct variants HHV-6A and HHV-6B have been identified. Despite a high degree of similarity in DNA, these two variants have distinct biological properties and are associated with specific pathological conditions. In November 2018, the NVRL replaced its laboratory developed HHV-6 assay with a commercial assay which distinguishes between HHV-6A and HHV-6B. This study looks at the prevalence of these two HHV-6 variants in Ireland from Nov 2018-Apr 2019”

Method: "Samples (CSFs, oral fluid, plasma and throat swabs) from clinically relevant patients were tested by the HHV-6 PCR kit*. All CSF samples from patients <3 years and oral fluids that were measles negative by PCR were also reflexed for HHV-6 testing."

Results: “A total of 851 samples were tested for HHV-6 between Nov 2018 and Apr 2019. Of these, 177/851 (20%) were positive for HHV-6 and 171/177 (97%) were identified as HHV-6B. Both variants were found in all sample types tested. CSF samples accounted for 20% of positives. The virus was detected equally between males and females. Of the 152 measles negative samples tested, 74% (112/152) were HHV-6 positive.”

Conclusions: “HHV-6B is the dominant HHV-6 virus detected in Ireland. HHV-6 is a significant causative agent in neurological disease in children under 3 years. This study also highlighted the importance for further testing of measles negative samples.”

* Altona RealStar®
Aim: “Aim of the study was to check Culex spp. mosquitoes collected in Greece for flaviviruses and alphaviruses in order to estimate the infection rate and to identify potential endemic foci.”

Method: “During May to October 2018, 17,470 Culex spp. were collected using CO₂ traps from all prefectures of Central Macedonia region in Greece. Based on the location and date of collection, the mosquitoes were grouped into pools (n=229), and RNA was extracted. Two nested RT-PCRs targeting flaviviruses and alphaviruses were applied, while one West Nile virus (WNV)-specific PCR was applied to the flavivirus positive pools. PCR products were sequenced, and phylogenetic analysis followed.”

Results: “Flaviviruses and alphaviruses were detected in 12 (5.2%) and 8 (3.5%) mosquito pools, respectively. Ten pools were positive for WNV lineage 2 clustering into the Central European/Hungarian subclade, while in 2 pools the flavivirus was insect-specific virus. The highest minimum infection rate was identified in Thessaloniki prefecture, where several human cases of WNV infection occurred in 2018. The first WNV-positive pool was detected two weeks prior the report of the first human cases in the region. All alphaviruses were Eilat virus, which is an insect-specific alphavirus.”

Conclusions: “Results of study suggest that testing mosquitoes can serve as early warning system for raising awareness among clinicians and public health authorities, for implementing prevention measures and for strengthening the mosquito control strategies. Furthermore, phylogenetic analysis is helpful for understanding the spread and evolution of the virus.”

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**[P162] EVALUATION OF A REAL-TIME RT-PCR KIT FOR DETECTION OF WEST NILE VIRUS**

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**Aim:** “West Nile virus (WNV) causes to humans an asymptomatic, mild or neuroinvasive infection. Aim of the study was to evaluate a real-time RT-PCR kit for diagnosis of WNV infection.”

**Method:** “Viral RNA was extracted from 63 samples (31 whole blood, 32 urine) taken 3-16 days after symptoms’ onset from 43 Greek patients with serologically diagnosed WNV infection. For 20 patients both blood and urine samples were tested. Samples were pre-tested by an in-house RT-nested PCR, previously designed for WNV detection and lineage identification, and by WNV RT-PCR Kit 2.0*.”

**Results:** “The WNV RT-PCR Kit 2.0* successfully detected WNV in 49 samples, while the in-house PCR detected the virus in 33 samples. The virus belonged to WNV lineage 2. Thirteen samples were negative by both PCRs; one urine sample was positive only by the in-house PCR (possibly due to urine degradation during storage). The less sensitivity of the in-house PCR can be explained by the relative large size of the PCR product. The WNV RT-PCR Kit 2.0* was also used to compare the viral load based on Ct value between blood and urine; in most cases the viral load was higher in blood.”

**Conclusions:** “Data indicates that WNV RT-PCR Kit 2.0* is a useful tool for diagnosis of WNV infection with high specificity allowing the detection of low-level viremia and viruria, and can be used for diagnostics and study of virus kinetics.”

**Funding:** “Altona provided the test kit.”

* RealStar®, altona Diagnostics, Germany)
PARVOVIRUS 4 AND HUMAN BOCAVIRUSES INVOLVEMENT IN INFLAMMATORY NEUROLOGICAL DISEASES

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Aim: The aim of the study was to determine the relationship between parvoviral infections and neurological diseases in humans.

Method: Cell-free blood plasma and cerebrospinal fluid (CSF) specimens were collected from clinically confirmed meningitis/meningoencephalitis (M/ME) patients as well as healthy controls. IgG and IgM class antibodies against parvoviral antigens in human plasma and CSF samples were measured using indirect EIA format using yeast-generated HBoV1-4 and hPARV4 VP2 VLPs. Specific antibodies against HBoV1 and HBoV2-4 were determined using a competition EIA format. Plasma was incubated with HBoV1 or HBoV2-4 VP2 proteins in order to eliminate cross-reacting antibodies. Overall, 131 plasma samples (81 samples of patients with M/ME and 50 samples of healthy individuals) and 24 CSF specimens of patients with M/ME were analysed.

Results: Seroprevalence of IgG/IgM antibodies against hPARV4 and IgM antibodies against HBoV1 was not significantly different in controls and patients. However, IgG antibodies against HBoV1 were found in M/ME patients more often than in control group (p<0.05). Anti-HBoV1 IgG antibodies were also found in CSF of 5 patients. There were no patients who had IgM antibodies against hPARV4 VP2 or HBoV1-4 VP2 in their CSF.

Conclusions: The absence of acute parvoviral infection markers in M/ME patients suggests limited involvement of parvoviruses in neurological diseases in humans. Further investigation using molecular virus diagnostic methods could help determine the role of parvoviruses in inflammatory neurological diseases.

Acknowledgements: This research was funded by a grant (No. TAP LLT-17-012) from the Research Council of Lithuania.
PRESENCE OF PARVOVIRUSES INFECTION MARKERS AMONG PATIENTS WITH ENCEPHALOPATHY

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Aim: “To determine the frequency of parvovirus B19 (B19V), human bocaviruses 1-4 (HBoV1-4) and parvovirus 4 (hPARV4) infection markers among patients with encephalopathy.”

Method: “31 patients with encephalopathy and 50 blood donors (control group) were examined. The presence of HBoV1-4, B19V and hPARV4 genomic sequences were detected in DNA samples isolated from peripheral blood using PCRs and parvovirus-specific IgG and IgM class antibodies determined in blood plasma by indirect EIA using yeast-generated HBoV1-4 and hPARV4 VP2 virus-like particles, and by RecomWell Parvovirus B19 IgG and IgM test kits.”

Results: “HBoV1-4 genomic sequences were detected in 29% of patients and 28% of controls (p>0.9999) but B19V genomic sequence - in 3.2% and 6%, respectively (p>0.9999). Presence of hPARV4 genomic sequence was not found. 64.5% of patients and 34% of controls were HBoV1 IgG positive and 12.9% of patients and 8% of controls – HBoV1 IgM positive. HBoV2-4-specific IgG class antibodies were detected in 25.8% of patients and 30% of controls, but HBoV2-4 IgM in 3.2% of patients and 8% of controls whereas hPARV4 IgG - in 16.1% of patients and 10% of controls, but IgM - in 3.2% and 4%, respectively. 83.9% of patients and 74% of controls were B19V IgG positive, but 3.2% of patients and none of controls IgM positive.”

Conclusions: “Results show that parvovirus-specific infection markers are present among patients with encephalopathy as well as control group. Further research on post-mortem brain tissues is needed to evaluate the role of parvoviruses in the pathogenesis of encephalopathy.”
A recent increase of tick-borne encephalitis cases in France: 2013-2018

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Aim: Tick-borne encephalitis (TBE) is historically uncommon in France and confined to the Far-Eastern part of the country. However, in view of the consistent increase in TBE cases in Europe, we reviewed the epidemiological and clinical data of recent French cases.

Method: From 2013 to 2018, 3005 sera or cerebrospinal fluid samples from 2675 patients were tested by ELISA (Serion classic TBE virus; TBE Enzygnost Siemens). Clinical and epidemiological data were collected and analyzed according to the TBE IgM and IgG status.

Results: Ninety (3.36%) patients were TBE positive according to the ECDC criteria, including 71% from Eastern France, 9% from the Alpine region and 1% from the Auvergne region. Tick bite was the main exposure factor. Since 2016, the number of confirmed cases per year has doubled (10 to 20). 64% of patients presented meningoencephalitis, 27% meningitis and 9% had no neurological symptoms. TBE serology was performed a mean 3 days following hospitalization: from 6 days in 2015 to 2.5 days following 2016. The mean hospitalization duration was 9.5 days (0 to 60). Forty-five patients were followed-up after primary infection (range: 15 days to 10 months). Among them seventeen showed an incomplete recovery (residual tremor (n=4), residual facial palsy (n=1), motor deficits (n=3), cognitive complaints (n=8), persistent asthenia (n=4).

Conclusions: An upsurge in TBE confirmed cases was observed since 2016, with an expansion of the areas at risk for TBE. Physicians should be aware of this change in TBE epidemiology.
The most detected target HHV-6 in CSF by syndromic molecular panels, is it real?

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Aim: Human herpesvirus 6 (HHV-6) has been the most frequent positive target with ME panel* at our hospital. So, we aimed to investigate the clinical significance of HHV-6 as cause of encephalitis.

Method: CSF samples with suspected meningoencephalitis were included at Marmara University Hospital, Department of Microbiology from 30 October 2017 to 30 April 2019. ME Panel* was performed and quantitative kit** was used for positive HHV-6 results with ME Panel*.

Results: A total of 731 samples were included, of whom 78 (10.7%) tested positive for at least one target and 21 (26.9% of positive cases) tested positive for HHV-6. Five patients were adults, 15 were infants and one was 18-month baby. Most common symptoms were fever and febrile convulsion in pediatric patients; altered mental status in adults. HHV-6 viral load test was performed in ten patients and HHV-6 DNA was detected in nine. After infectious disease specialist consultation only one patient was evaluated as encephalitis. In this patient Listeria monocytogenes and HHV-6 were detected in the ME Panel*, Listeria monocytogenes was isolated from CSF and also HHV-6 with high viral load was detected in both CSF and plasma samples (respectively; 619340 copies/mL and 35681 copies/mL).

Conclusions: Although HHV-6 was the most detected target with ME panel* in our patient group, the majority of these results were unlikely to be encephalitis with the evaluation of clinical, radiological and laboratory data.

* FilmArray Meningitis/Encephalitis (ME)
** HHV-6 Elite MGB® kit (Elite in Genius, Elitech Group, Italy)
Aim: “The objective of this study was to genetically characterise circulating HPeVs in Ireland between 2015 and 2017 in patients under 3 years of age and assess the clinical associations attributed to specific HPeV genotypes.”

Method: “HPeV genotyping, based on the VP1 capsid protein, was performed on 54.0% of all positive cases. Clinical and demographic information was also collected.”

Results: “Laboratory-confirmed HPeV infections were identified in 215 patients which represented a positivity rate of 3.9% (n=215/5450). A total of 116 patient samples were successfully genotyped. The most common HPeV genotypes in circulation during the study period were HPeV-3 (n=71/116, 61.2%) and HPeV-1 (n=25/116, 21.6%). Peaks of HPeV activity occurred each year during the summer and autumn months. There was a greater number of HPeV infections during the 2016 (n=117) period compared to 2015 (n=36) and 2017 (n=62). Clinical information was available for 89 children; 34.8% (n=31) presented with sepsis-like syndrome, 19.1% (n=17) with fever/irritability, and 14.6% (n=13) with neurological involvement. HPeV-3 was significantly more often associated with sepsis-like syndrome than any other HPeV genotype and was also significantly more likely to occur in patients under 3 months old.”

Conclusions: “This study has provided baseline genotypic surveillance data of HPeV in Ireland for the first time. The data highlight that HPeV, in particular HPeV-3, is an important cause of illness in children. The future availability of HPeV genotyping data will have beneficial impact on clinical management and provide real-time information to inform public health policy.”
Aim: To present a difficult diagnostic meningoencephalomyelitis case which ultimately revealed pegivirus infection using genomic based techniques.

Method: Case report and literature review

Results: A 28-year-old male presented with bilateral deafness and left optic neuropathy. Significant past medical history included autoimmune enteropathy since childhood. This had been under control with steroids and tacrolimus. In 2016 he developed an invasive fungal infection requiring colectomy and a significant reduction in immunosuppression. He subsequently had a flare and was treated with Vedolizumab. Within weeks, he developed quadriparesis with urinary retention. MRI brain showed significant meningoencephalomyelitic imaging syndrome. Brain biopsy revealed non-specific inflammatory changes, CSF was inflammatory yet all targeted and culture-based infection screens were negative. Flow cytometry and CSF cytology were conclusively negative. He was treated empirically for tuberculosis, and with IV methylprednisone with only transient improvement. Given all other diagnostic avenues had been explored a brain biopsy was sent for metagenomic assessment. This ultimately revealed a large number of reads for pegivirus. The significance in this scenario is difficult to assess given the ubiquitous nature of the virus. As it was the only pathogen found it is possible to consider that this may have been the cause of his meningoencephalitis. A literature review concludes that there have been a small number of associations with pegivirus and encephalitis.

Conclusions: This case highlights the emerging possibility of genomic based techniques in diagnosis of infectious diseases which have remained elusive to other diagnostic techniques. This also brings its own challenges as highlighted.
Aim: Hepatitis A virus (HAV) is the commonest cause of acute viral hepatitis. In industrialized countries, most of population is at risk of HAV infection until advanced age of 50 years old. Rare cases of autoimmune hepatitis have been reported after acute viral HAV infection. Mechanism of such disease is not well understood.

Method: A 57-year-old women developed acute autoimmune hepatitis (AIH) after prolonged hepatitis A infection.

Results: Virological diagnosis was based on IgM detection by electrochimiluninescence immuassay (Cobas, Roche) and RNA virus amplification by real time PCR at 6.08 log UI/ml. RNA sequencing of VP1/VP2 target region detected a genotype IA virus. Two month later, re-increases of jaundice and liver enzymes were observed, RNA-HAV decreased but was still detectable in serum. HAV avidity at 73% confirmed a prolonged HAV infection. However, gammaglobulins and IgG levels were higher than those at admission 26.85 g/l and 20.7 g/l respectively. Serological testing of antinuclear antibodies and specific auto antibodies were negative. Liver biopsy made the histological diagnosis of AIH superimposed on prolonged acute hepatitis A infection. A favorable response to corticosteroid treatment was obtained and no further elevation of transaminases was observed after corticosteroid treatment cessation.

Conclusions: The hypothesis of excessive immune reaction to infection may lead to onset of AIH need to be confirmed. So, the clinical follow-up of acute hepatitis A infection can improve the early diagnosis and management of AIH in case of differed relapse of cytolytic hepatitis.
Figure 1: Evolution of serum transaminases, total bilirubin and RNA-HAV
HEPATITIS B VIRUS S GENE MUTATIONS AT PATIENTS WITH OCCULT HEPATITIS B VIRUS INFECTION

Bulent Cakal

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Aim: The aim of this study was to detect mutations S gene region of Hepatitis B virus (HBV) in patients with occult HBV infection (OBI).

Method: Determined of patients with OBI: This study was used to liver tissue samples had been obtained from HBsAg-negative patients. OBI was defined as HBV DNA positivity in 2 or more different viral genomic regions by nested PCR. A total of 16 subjects with confirmed OBI infection were recruited to the study. Nested PCR was performed to amplify of HBV S-gene (Table 1). Sequence analysis of purified products was performed with a Genetic analyzer (Becman Coulter). SF2 and SR2 also SF3 (Table 1) was used for sequencing.

Results: Only one patient T125M and P127T mutations within the major B-cell epitope cluster (amino acid 124–147; referred to as “a”-determinant) were detected of major hydrophilic region (MHR) of hepatitis B surface antigen (HBsAg). In the downstream of the MHR (HBsAg c-terminus) was detected to immune escape mutations in 10 patients; T189I, S204N, S207R, S207N, S210N, L213I, L216F, P217L (Table 2).

Conclusions: According first results of this study is that OBI could be associated with weak immune response due to immune escape mutations.
### Table 1: Primers used for amplification of Pre-S Gene

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5' - 3')</th>
<th>Polarity</th>
<th>Domain</th>
<th>Positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBVSF1</td>
<td>CCTGCTGGTGGCTCAGTTCA</td>
<td>Sense</td>
<td>PreS</td>
<td>56-76</td>
</tr>
<tr>
<td>HBVSR1</td>
<td>GCTAGGAGTTCGCAGTATGG</td>
<td>Antisense</td>
<td>PreS</td>
<td>1286-1266</td>
</tr>
<tr>
<td>HBVSF2</td>
<td>CATGGAGAACATCACATCAGG</td>
<td>Sense</td>
<td>PreS</td>
<td>155-174</td>
</tr>
<tr>
<td>HBVF2</td>
<td>CGTTGACAGACTTTCAATCAAT</td>
<td>Antisense</td>
<td>PreS</td>
<td>995-973</td>
</tr>
<tr>
<td>HBVF3</td>
<td>TGCTGCTATGCCTCATCTTC-</td>
<td>Sense</td>
<td>PreS</td>
<td>414-433</td>
</tr>
</tbody>
</table>

### Table 2: HBsAg mutations at patients with occult hepatitis B virus infection

| OBI Patient D ayw2 Ayw3 T125M P127T T189I S204N S207R S207N S210N L213I L216F P217L | HBsAg C-terminus/T-cell epitopes 'a'determinant |
|----------------------------------|----------------------------------|----------------------------------|--|---|---|---|---|---|---|---|---|---|
| OBI 1 HCV                        | +                               | +                               | +                          | +                          | +                          |
| OBI 2 HCV                        | +                               | +                               | +                          | +                          | +                          |
| OBI 3 HCV                        | +                               | +                               | +                          | +                          | +                          |
| OBI 4 Cryptogenic                | +                               | +                               | +                          | +                          | +                          |
| OBI 5 Cryptogenic                | +                               | +                               | +                          | +                          | +                          |
| OBI 6 HCV                        | +                               | +                               | +                          | +                          | +                          |
| OBI 7 Cryptogenic                | +                               | +                               | +                          | +                          | +                          |
| OBI 8 Cryptogenic                | +                               | +                               | +                          | +                          | +                          |
| OBI 9 Cryptogenic                | +                               | +                               | +                          | +                          | +                          |
| OBI 10 Cryptogenic               | +                               | +                               | +                          | +                          | +                          |
| OBI 11 HCV                       | +                               | +                               | +                          | +                          | +                          |
| OBI 12 Cryptogenic               | +                               | +                               | +                          | +                          | +                          |
| OBI 13 Cryptogenic               | +                               | +                               | +                          | +                          | +                          |
| OBI 14 Cryptogenic               | +                               | +                               | +                          | +                          | +                          |
| OBI 15 Cryptogenic               | +                               | +                               | +                          | +                          | +                          |
| OBI 16 Cryptogenic               | +                               | +                               | +                          | +                          | +                          |
Aim: HAV infection is often asymptomatic in the early years of life, but the severity of illness increases with age. HEV is a substantial cause of illness and mortality worldwide, particularly among pregnant women. The burden of hepatitis A (HAV) and hepatitis E (HEV) infections are unknown in Turkestan Region, Kazakhstan. We aimed to carry out providing an overview of the seroepidemiology of HAV and HEV in Turkestan Region and to evaluate the association of HAV and HEV infections with age.

Method: Our study included a total of 878 participants 512 (58.3%) female and 366 (41.7%) male, aged between 5 and 75. We divided our participants into 5-12, 13-18, 19-40, 41-65, 66-75 age groups and tested the serum samples for anti-HAV and anti-HEV antibodies by chemiluminescent microparticle immunoassay (CMIA) and ELISA respectively.

Results: The seroprevalence of HAV and HEV were 93.8% and 5.5%, respectively. Most people in our study were HAV positive until the age of 40. The highest number was observed in 19-40 (45.8%) age group among anti-HAV positive samples. No positivity was observed for anti-HEV antibodies until the age of 13, while the highest number of positive samples were identified in the 41-65 (70.8%) age group.

Conclusions: Our results showed the seroprevalence of anti-HAV is high. It was seen that anti-HEV positivity in our study was introduced with fertility age. We believe that the high prevalence of HAV is associated with undeveloped sewage systems and the use of well water as drinking water. Although HEV infection rates are high in Asia; according to our results, it is low in Turkestan region, as in some other countries.
Aim: “Men who have sex with men (MSM) are targeted for hepatitis B virus (HBV) vaccination, in the Netherlands, since they account for approximately a third of chronic HBV cases among men. HBV testing rates have increased recently due to recommendations for frequent HIV/STI testing among MSM. MSM enquiring pre-exposure prophylaxis (PrEP) for HIV prevention have HBV tests, which could lead to higher HBV testing and vaccination rates. We investigated the impact of increased HBV vaccination, testing and treatment on HBV transmission.”

Method: “We developed a mathematical model for HBV transmission among MSM. We examined the following changes: (a) increased vaccination rate from 2% currently to 4% among high-risk MSM; (b) increased HBV testing every three months among high-risk MSM (every 6-18 months currently); (c) increased HBV testing and vaccination rates by 0.5% among high-risk MSM, due to PrEP.”

Results: “From the model, the HBV incidence in 2020 was calculated at 44 (interquartile range (IQR), 36-50) new HBV infections per 100,000 MSM. HBV incidence can be reduced by 56% (IQR, 53-60%) or 11% (IQR, 9-13%), 20 years after increasing vaccination or testing rates, respectively. Assuming that 0.5% of high-risk MSM may have HBV tests due to PrEP, resulted in a 20% (IQR, 18-22%) reduction in HBV incidence.”

Conclusions: “Increased HBV testing can contribute to reducing HBV transmission, but major declines in HBV incidence can be achieved by increasing HBV vaccination rates among high-risk MSM. PrEP could indirectly result in reduced HBV incidence, by increasing HBV vaccination and testing rates.”
EVALUATION OF THE SENSITIVITY OF REALSTAR® HEV RT-PCR KIT 2.0 WITH THREE COMMONLY USED EXTRACTION METHODS

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Aim: Hepatitis E virus (HEV) is a pathogen that causes hepatitis worldwide. A substantial increase in acquired HEV cases is observed across Europe where HEV genotype 3 infections, originating from animal reservoirs, are predominant and have become a common cause of acute viral hepatitis. The aim of this study is to show the high sensitivity of the RealStar® HEV RT-PCR Kit 2.0 for HEV genotypes 1 to 4.

Method: Serial dilution of the 1st WHO International Standard for Hepatitis E Virus, genotype 3a (PEI code: 6329/10) in HEV negative human EDTA plasma were tested. Probit analysis was done with StatsDirect statistical software. The LoD was confirmed for the genotypes 1, 2 and 4 (1st WHO International reference panel for Hepatitis E Virus genotypes (PEI code: 8578/13). Three different extraction methods (QIAamp Viral RNA Kit, EasyMag extraction and MagNaPure 96) were used. Additionally, 5 different real-time PCR instruments (LightCycler® 480 Instrument II; CFX96™ Real-Time PCR Detection System; ABI Prism® 7500; Rotor-Gene® Q5/6; VERSANT® kPCR Molecular System AD,) were compared with respect to equivalent performance.

Results: Depending on the extraction method, the Limit of Detection varied between 35 IU/ml to 49 IU/ml. The LoDs were confirmed for all genotypes.

Conclusions: The RealStar® HEV RT-PCR Kit 2.0 allows sensitive detection and quantification of HEV RNA in human EDTA plasma, independent of the extraction methods and real-time PCR instruments used.
PREVALENCE OF HEPATITIS A VIRUS STRAINS LINKED TO EUROPEAN OUTBREAK IN MEN HAVING SEX WITH MEN (MSM) IN THE CZECH REPUBLIC.

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¹National Institute of Public Health, Prague, Czech Republic
²Veterinary Research Institute, Brno, Czech Republic

Aim of present study was to determine strains of hepatitis A virus (HAV) found in the Czech Republic since the year 2016.

Method: Sequence and phylogenetic analyses of viruses originating from clinical samples of patients suffering from hepatitis A were performed. Region VP1/2A of HAV genome was sequenced following recommended protocol of HAVNET.

Results: Sequences of specific part of HAV genome originating from a total of 354 patients were analysed; 175, 110 and 65 cases in years 2016, 2017 and 2018, respectively and additional 4 cases until March 2019. Thirty (8.5 %) of obtained HAV sequences revealed ≥99.3% identity to one of the three HAV genotype IA outbreak strains affecting predominantly MSM. Ten sequences were identical to strain RIVM_HAV_16-090, 3 with 1nt and 1 with 2nt difference. Three sequences were identical to strain V16_25-801 and 1 with 2nt difference. Five sequences were identical to strain VRD_521_2016 and 6 with 1nt difference. From 30 patients infected with these strains were 22 males, 2 females, in 6 sex was not reported. Sixteen of MSM strains associated cases were diagnosed in Prague.

Conclusions: The strains of HAV related to European MSM outbreak were detected in 8.5 % of hepatitis A patients analysed in the Czech Republic.

This work was supported by grant No.17-31921A of Agency for Medical Research (AZV) of Ministry of Health of the Czech Republic.
EVALUATION OF ALINITY M, REALTIME AND COBAS 8800 HEPATITIS B VIRUS (HBV) VIRAL LOAD ASSAYS ACROSS A-H HBV GENOTYPES

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¹Abbott Molecular Inc, Des Plaines, United States

Aim: Treatment guidelines recommend the use of HBV DNA as one of the treatment indicators. Currently there are several fully automated commercially available HBV viral load assays. These assays differ in their design features, analytical performance characteristics and platform automation. This study compared performance of the two recently approved assays to that of the well-established RealTime HBV assay.

Method: Assay performance was evaluated across clinical samples from HBV genotypes A-H. Comparison between assays was evaluated by assessing: assay correlation, mean bias between assays, percentage of samples with <0.5 Log IU/mL difference, and percentage of samples with > 0.5 to <1.0 Log IU/mL difference.

Results: Study results are summarized in the table below.

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>RealTime - Alinity m N=279</th>
<th>Alinity m - cobas 8800 N=125</th>
<th>RealTime – Cobas 8800 N=125</th>
</tr>
</thead>
<tbody>
<tr>
<td>R²</td>
<td>0.993</td>
<td>0.994</td>
<td>0.996</td>
</tr>
<tr>
<td>Mean Bias (Log IU/mL)</td>
<td>-0.18</td>
<td>0.09</td>
<td>-0.01</td>
</tr>
<tr>
<td>% Samples Different by &lt; 0.5 Log IU/mL</td>
<td>97.1</td>
<td>91.2</td>
<td>95.2</td>
</tr>
<tr>
<td>% Samples Different by &gt; 0.5 to &lt;1.0 Log IU/mL</td>
<td>2.9</td>
<td>8.8</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Conclusions: Clinical assessment of the newly approved Alinity m HBV assay demonstrated good correlation and performance versus two comparator assays. Correlation coefficient ranged from 0.993 to 0.996. The observed mean bias between any two assays ranged from -0.175 to 0.09 log IU/ml.
[P177] HBV VL, A NEW TOOL FOR SIMPLIFIED HBV VIRAL LOAD TESTING AND DISEASE MANAGEMENT

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Aim: “We compared the analytical performance of the new HBV VL assay* to the HBV VL assay**. The workflow of each assays has been evaluated.”

Method: “For the precision, 4 specimen have been prepared (0, 1.64, 6.09 and 7.92logIU/mL). For the method comparison (MC), 85 specimens from infected patients were tested. The patient monitoring has been carried out on 80 serial samples collected from 24 patients. Inclusivity has been assessed on 4 genotypes. The turn-around-time (TAT), the hands on-time (HoT) have been determined on both assays.”

Results: “The MC showed a high correlation (R²=0.99, slope=1.04) and a Mean Difference of -0.015 (CI 95%=-0.05-0.02). Linearity on the genotypes shows an R² >0.95. Precision gave a standard deviation <0.1logIU/ml. The method** required 7h to process all the samples (1 week activity), versus 3h21 with method*. The HoT required by the assay* is lower (36mn) than the HoT for assay** (54mn). In our laboratory the test** is performed twice a week and the mean TAT is 89h34mn. The assay* showed a TAT of 3h21mn.”

Conclusions: “A good correlation was observed between both assays. Inclusivity and linearity showed very good performances. The PCR steps of HBV VL assay* are all performed onto a single platform***. It reduces consequently the complexity of the process and the labor time. HBV VL assay* allows continuous loading of samples, which eliminates the need for batching. It also allows urgent samples testing, leading to a significative improvement of our productivity.”

* Cepheid Xpert, ** Abbott m2000, ***GeneXpert
Aim: HCV genotyping is important for monitoring the changes in HCV epidemiology. In this study, we aimed to evaluate five years HCV genotyping results among intravenous drug users (IVDU) and HCV infected patients unrelated to drug use, retrospectively.

Method: Plasma samples of 720 patients which were sent to Akdeniz University Hospital Central Microbiology Laboratory, Antalya, Turkey for HCV genotyping between January 2014-March 2019 were analysed. NLM (Milan, Italy) kit was used to determine 7 types of HCV. HCV RNA was extracted with Virus Mini Kit* (Hilden, Germany); amplified with PCR** (Hilden, Germany) according to manufacturer’s instructions.

Results: Study group was consisted of 454 non-drug users (51.3% male; median age: 56.5±16.06 years) and 266 IVDU (93.2% male; median age: 25±6.82 years). The IVDU group was significantly younger and had higher proportion of males (p<0.05). Genotype 1b was detected in 62.3% (283/454), 1a in 20.5% (93/454), 3a in 9.5% (43/454) of non-drug users; whereas, genotype 1a in 54.1% (144/266), 3a in 31.6% (84/266), 1b in 5.3% (14/266) of IVDU. Genotypes 1a and 3a were common in IVDU and males, while 1b was more common in non-drug users and females (p<0.05). 92.3% of genotype 4c/d and 80% of genotype 2b patients were IVDU.

Conclusions: The most common HCV genotype was 1a among intravenous drug users and the most common HCV genotype was 1b among non-drug users. Genotype 3a and 1a were significantly increased compared to years (p<0.05).

* Qiagen EZ1
** Qiagen Rotorgene 6000 Real-Time
EVALUATION OF A NEW RAPID AND FULLY AUTOMATED MOLECULAR ASSAY FOR THE QUANTIFICATION OF HBV DNA ON DRIED BLOOD SPOTS

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²Pontchaillou University Hospital, University Rennes, Virology, Rennes, France

Aim: Hepatitis B virus (HBV) infection remains a public health problem, particularly in resource-limited countries. HBV-DNA testing should be expanded. Dried blood spots (DBS) are a practical alternative to plasma. This study aims to evaluate the quantification of HBV-DNA on DBS using a new rapid molecular assay on fully integrated and automated system¹.

Method: Non-infected whole-blood (WB) samples were spiked with high HBV-DNA plasma (genotype-E). Using calibrated tainted WB, each DBS was prepared by 50µL deposition of pure or serial 1:10 dilutions on pre-cut 12mm paper card. Spots were dried at room temperature (RT) and stored away from light. After 7 days, 5 paired plasma-DBS were analyzed. HBV-DNA stability on DBS was evaluated after 31 days storage at RT.

Results: HBV-DNA from prepared dilutions ranged from 2.55 to 7.55 and 2.75 to 7.49 log IU/mL in plasma and in 7 days RT-stored DBS, respectively. An excellent correlation (R²=0.99, P<0.0001) was found between plasma and DBS. Bland-Altman analysis revealed a mean difference measurement of -0.10 log IU/mL between DBS and plasma. Lower limit of quantification on DBS was evaluated at 2.60 log IU/mL. In addition, DBS storage for 7 or 31 days did not significantly influence HBV-DNA quantification.

Conclusions: These results indicate that fully automated molecular diagnostic systems could accurately quantify HBV-DNA from DBS. Such strategy could improve accessibility to this key marker for the management of HBV-infected patients, particularly in resource-limited countries where easy-to-use solutions are already implemented.

¹Xpert HBV Viral Load RUO assay on GeneXpert system (Cepheid).
Aim: The objective of this study was to characterize performance of the HCV Test* across key performance metrics on the systems.

Method: Analytical studies were performed using plasma and serum to characterize various analytical parameter such as - sensitivity, cross-reactivity, inclusivity, interfering substances, cross-contamination, specimen stability, and turnaround time (TAT).

Results: The HCV Test*0 has a sensitivity of 7.5 IU/mL and 8.0 IU/mL with LLoQ of 7.7 IU/mL and 8.4 IU/mL in plasma and serum respectively using the 5th WHO International Standard for HCV. The HCV Test* demonstrated excellent linearity across a 7-log dynamic range (R2>0.99) as well as precision across 12 days with maximum overall deviation of 0.26 Log10 IU/mL at lowest level tested. The HCV Test* showed equivalent detection across all relevant HCV genotypes and a time to first result of ~80 min. No cross-reactivity or interference was observed against a panel of 33 pathogens and common interfering agents tested. The method correlation study performed using 646 clinical remnant plasma and serum specimens across two different systems** demonstrated excellent concordance with the respective reference test as indicated by a slope of 1.0 and intercept of less than 0.3.

Conclusions: The Test* is an extremely easy to use, rapid, automated test for the sensitive and accurate monitoring of HCV viral load.

*NeuMoDx, **NeuMoDx 96 and 288
Hepatitis E virus (HEV) is one of the most common causes of acute hepatitis in the world, disproportionately affecting low-income countries. Proper diagnostics of HEV in these areas is hampered by logistic difficulties in taking and storing blood samples for testing. Dried blood spots (DBS) are a less invasive, more robust, and cheaper alternative to plasma or serum specimens. The aim is to optimize and validate an ELISA method for detection of anti-HEV IgG with DBS.

Method: A previously validated ELISA method (HEV IgG from Wantai) was optimized for DBS (Whatman 903 filter paper) by testing plasma and DBS samples from HEV-negative donors spiked with HEV IgG (World Health Organization (WHO) 95/584).

Results: The dilution necessary to avoid false positives was 6 times higher in DBS compared to plasma. Limit of detection was 0.6 WHO-units (WU)/ml for DBS and 0.3 WU/ml for plasma. The average coefficient of variation in optical density above cut-off (OD/CO) was 25 in plasma and 48 in DBS. The average difference in OD/CO between plasma and DBS was 3.1. Sensitivity of DBS compared to plasma was 96 in samples above 0.6 WU/ml and specificity was 93.

Conclusions: HEV IgG ELISA results from DBS samples correlated well with plasma samples and the limit of detection using DBS is sufficient for most clinical samples. The need for higher dilution in DBS is likely due to hematocrit and matrix effects.

Acknowledgements: This study was financed by the Norwegian Research Council, project number: 248143/H10
[P182] HEPATITIS B SURFACE ANTIGEN VARIANTS AMONG CHRONIC HEPATITIS B INFECTIONS IN SOUTH INDIA

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1Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India
2Jipmer, Puducherry, India

Aim: ”Hepatitis B (HBV) prevalence in India ranges from 2 – 8%. Universal immunization for HBV was introduced only in 2007 – 08. This study aims at unraveling the natural variations in HBV in unvaccinated individuals in South Indian population”

Method: “A total of 70 chronic HBV infected patients (median age 30.5 years) in different stages of disease progression were included in the study. A 469 bp segment of S gene was amplified and sequenced. The amino acid substitutions were analysed using web based program from Max Planck Institute.”

Results: “Three genotypes (Gt); namely D (n=66), A (n=3) and C (n=1) were detected. In GtD, subtype G2 was predominant (n=64). Escape mutation A128V was observed in 60 isolates. Other escape mutants observed were 120S, 120T, 133I, 134N, 144E and 147S; of which 144E is a known vaccine escape mutation. Two isolates carried triple escape mutations. Significance of T118V surface antigen variant detected in 61 isolates is not known. Putative drug resistance mutation H126R in reverse transcriptase (rt) gene was observed in 63 samples. Silent rt mutations N53D (n=24) and Y54H (n=34) were also detected. Established drug resistance mutations in rt gene were not detected.”

Conclusions: ”Naturally occurring escape mutant and putative resistance mutations were detected in majority of isolates. Predominant mutation A128V is known for lower reactivity in diagnostic assays. Evolution of circulating strains with respect to vaccination and treatment needs to be followed”
EVALUATION OF COMMERCIALLY AVAILABLE HEPATITIS C VIRUS (HCV) VIRAL LOAD ASSAYS ACROSS DIFFERENT HCV GENOTYPES

Dan Toolsie¹, Brian Erickson¹, John Karavitís¹, Tetsuya Nakasatomi¹, Danijela Lucic¹, Shihai Huang¹

¹Abbott Molecular Inc, Des Plaines, United States

Aim: Approximately 80% of HCV infected patients are unaware of their infection and related liver disease because chronic viral hepatitis is generally asymptomatic until advanced liver disease develops. Once diagnosed, HCV RNA is the gold standard diagnostic tool used to confirm infection as well as monitor the efficacy of antiviral therapy. Current HCV RNA assays differ in their design features, analytical performance characteristics and platform automation. Limited comparison is available across some of these newly approved assays and platforms. This study evaluated clinical performance of the most commonly and recently approved commercial assays across different HCV genotypes.

Method: Assay performance was evaluated across clinical samples from HCV genotypes 1-5. Comparison between assays was evaluated by comparing: assay correlation, mean bias between assays, percentage of samples ≤1.0 log IU/ml and percentage of samples > 1.0 log IU/ml.

Results: Study results are summarized in the table below.

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>RealTime - Alinity m N=124</th>
<th>cobas 8800 - Alinity m N=125</th>
<th>Hologic - Alinity m N=123</th>
<th>Cobas 8800 -RealTime N=125</th>
<th>Hologic - RealTime N=122</th>
<th>cobas 8800 - Hologic N=122</th>
</tr>
</thead>
<tbody>
<tr>
<td>R²</td>
<td>0.94</td>
<td>0.95</td>
<td>0.93</td>
<td>0.97</td>
<td>0.85</td>
<td>0.96</td>
</tr>
<tr>
<td>Mean Bias log IU/ml</td>
<td>-0.13</td>
<td>0.20</td>
<td>0.02</td>
<td>0.34</td>
<td>0.11</td>
<td>0.26</td>
</tr>
<tr>
<td>% Samples different by ≤1.0 log IU/ml</td>
<td>98.4</td>
<td>98.4</td>
<td>100</td>
<td>98.4</td>
<td>98.4</td>
<td>100</td>
</tr>
<tr>
<td>% Samples different by &gt; 1.0 log IU/ml</td>
<td>1.6</td>
<td>1.6</td>
<td>0</td>
<td>1.6</td>
<td>1.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusions: Clinical assessment of the newly approved Alinity m HCV assay demonstrated excellent correlation and performance vs other comparator assays across diverse HCV genotypes.
SYSTEMATIC REVIEW OF HEPATITIS E VIRUS PREVALENCE IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Aim: Hepatitis E virus (HEV) has been described as an emerging cause of chronic hepatitis in immunosuppressed patients, such as hematopoietic stem cell transplant (HSCT) recipients. We aimed to perform a systematic review of published data to evaluate HEV infection prevalence among HSCT recipients.

Method: Literature search was conducted in PubMed and Scopus databases without time, sample size or population restrictions. We used the Preferred Reporting of Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the MetaXL software for statistical analysis to estimate the overall prevalence of HEV infection according to the different diagnostic approaches (HEV RNA and anti-HEV IgM/IgG detection).

Results: A total of 7 manuscripts were included for data analysis. Significant variation was observed according to the diagnostic method: anti-HEV IgM/IgG seroprevalence was 12.0% (95% CI: 0.16-28.5); while, HEV RNA prevalence was 1.50% (95% CI: 0.70-2.60). Isolated anti-HEV IgM seroprevalence was 2.00% (95% CI: 0.30-4.50), and anti-HEV IgG was 11.4% (95% CI: 1.80-26.3).

Conclusions: HSCT recipients are at risk of HEV infection, and the detection of HEV RNA and/or anti-HEV IgM/IgG is advised. The overall prevalence of HEV infection in HSCT patients differ according to the
Aim: Although there is limited information gathered about importance of co-infection with different retroviruses, it is a well-known fact which should be thoroughly examined. Infection with HTLV-I/II occurs with variable frequencies across different populations and geographic regions. There is not any available population-based study defining the number of individuals affected by HIV and HTLV-I/II co-infection in Turkey. The aim of this study is to identify the infection rates of HTLV-I/II viruses in HIV seropositive patients enrolled at Gazi University Faculty of Medicine.

Method: A total of 48 serum samples from patients who are previously tested HIV-seropositive were included in this study. Serum samples were tested for HIV p24 antigen and antibodies to HIV-1/HIV-2 by chemiluminescent microparticle immunoassay (CMIA). RNA extraction was performed using a commercial kit. Detection and quantification of HIV RNA levels was conducted by Real-Time quantitative PCR method. The samples were screened for antibodies against HTLV-I/II using CMIA.

Results: The study population ranged in age from 6 to 60 years. Among the study population, 40(83.3%) and 8(16.7%) patients were male and female, respectively. Of these, 18(37.5%) patients had viral load of <1000 copies/mL, 10(20.83%) patients had viral load of 1000-10000 copies/mL, 20(41.67%) patients had viral load of ≥10000 copies/mL. We did not find any HTLV seropositive case.

Conclusions: Even though the co-infection has a significant effect on disease progression, testing for HTLV in HIV individuals is not routinely performed. More detailed further investigations should be carried out for prevalence estimates and assessment of clinical and biological significance of co-infection with retroviruses.
EVALUATION OF HIGH RISK HUMAN PAPILLOMA VIRUS GENOTYPES IN OUR REGION, ANKARA, TURKEY

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Aim: Human papillomavirus (HPV), is the major causative agent of cervical cancer. Fourteen subtypes of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) are detected in approximately 94.8% of cervical cancers. This study aimed to evaluate the distribution of high-risk genotypes among women in our region.

Method: Cervical samples collected by cervical brush* and sent to our laboratory between September 2018 and March 2019 were examined retrospectively. HPV DNA isolation was performed by automated extraction system** and amplification was performed by system*** with Bosphere high-risk hpv genotyping kit v1**** detecting 14 genotypes (type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

Results: Cervical samples of 125 female patients were evaluated. Of the patients evaluated 13 (17%), 83 (50%) and 29 (33%) were 18-25 years old, 26-45 years old and 46 years and older respectively. A total of 44 (35%) samples were HPV positive and 81 (65%) were HPV negative. Of the positive samples; 24 (55%) included single genotype, while 15 (34%), four (9%) and one (2%) included two, three and four different genotypes respectively. HPV16 was the most common genotype, whereas HPV 33 and 45 were the second most common genotypes.

Conclusions: Determination of high-risk HPV genotypes in our region is very important in terms of epidemiological research, screening and vaccination programmes in our country.

*(BD Surepath, BD Diagnostics, Heidelberg, Germany)
** Magnesia 16 (Anatolia Geneworks®, Turkey)
***Montania 4896 Real Time PCR System (Anatolia Geneworks®, Turkey)
****Anatolia Geneworks®, Turkey
Aim: “The aim of this study is to conduct molecular characterization and Phylogenetic study of Human Papillomavirus in Northern Nigeria.”

Method: “Cervical smear samples of 148 women were collected, of which 129 were analyzed for HPV DNA. HPV detection was conducted using MY09/11 and GP5+/6+ general consensus primers. HPV-positive samples were sequenced, and Single nucleotide polymorphism analysis was carried out to detect possible mutations. HPV variants relatedness was determined by phylogenetic analysis.”

Results: “HPV DNA was found in 34/129 of the samples giving a prevalence of 26.4%. A prevalence of 7.8% in subjects with normal cytology and 18.6% in those with squamous intra-epithelial lesion was found. HPV 16 have the highest prevalence of 26.9% out of 15 identified serotypes. A total of 14 single nucleotide polymorphisms were detected, two being synonymous, while 12 are nonsynonymous. It was found that 66.7% of the HPV 16 isolates belongs to the same lineage, with the remaining 33.3% belonging to African (C) lineage. A variant of HPV 16 and HPV 56 were found to cluster with lineage A1 (European), while a variant of HPV 6 and HPV 66 clustered with lineage B (African-1). Pairwise distance estimation of some sequences from this study and some reference sequence revealed a distance ranging from 0.0082 to 2.0994.”

Conclusions: “This study provides important data on HPV epidemiology, identification and characterization which will be fundamental in curbing the menace of HPV infection through the production of vaccines specific to HPV serotypes of Nigerian origin.”
[P188] ANALYSIS ON THE RESULTS OF HIV CONFIRMATORY TESTING USING WESTERN BLOT P31 BAND AND NUCLEIC ACID TEST (NAT)

Jin-Sook Wang¹, Hyo-Jung Sim¹, Chun Kang¹, Yoon-Seok Chung¹

¹Korea Centres for Disease Control & Prevention, Cheongju, Chungbuk, Korea, Rep. of South

Aim: The HIV confirmatory testing verifies positive results of HIV screening by combining experiments such as HIV antibody, antigen, neutralization test, western blot, and NAT. The 17 Provincial Institutes of Health and Environment (PIHEs) have used the HIV antibody, antigen and western blot method as a HIV confirmatory testing, while the Korea Centres for Disease Control and Prevention (KCDC) has added HIV antigen neutralization and NAT along with the three tests above to increase the detection of early HIV infections. This study aims to analyse the results of the HIV confirmatory testing conducted by the KCDC and the 17 PIHEs during 2015-2017.

Method: HIV western blot p31 was recognized as a marker that appeared 100 days after HIV infection. Therefore, western blot p31 band was used as the criteria for HIV infection period, and the HIV viral load changes according to patterns of HIV antigen and HIV antibody were analyzed.

Results: The results of the p31 reactive band were 27.4% (271/988) and 88.4% (5539/6266) respectively in the KCDC and the PIHEs. The group (n=124) with positive results for both HIV antigen and NAT showed all p31 non-reactive in western blot.

Conclusions: The results from the KCDC showed that 72.6 percent of the HIV-positive results were estimated to be the initial HIV cases of western blot p31 non-reactive. This demonstrated early diagnosis of HIV infections by applying HIV NAT and antigen neutralization tests to the HIV testing algorithm.

Acknowledgement: This study was funded by HIV/AIDS & STD (4800-4842-304) from the KCDC.
Aim: In 2017, 940 000 people died from HIV-related causes globally. There were approximately 36.9 million people living with HIV at the end of 2017 with 1.8 million people becoming newly infected in 2017 globally. According to FDA and CDC, virologic assays directly detecting HIV must be used to diagnose HIV infection in infants and children younger than 18 months. The HIV assays should demonstrate the highest possible standard of clinical performance. The aim of this study was to evaluate clinical performance characteristics of the innovative PCR assay* intended for diagnosis of HIV-1 virus from clinical samples.

Method: The clinical validation was performed on 628 plasma samples in total, 128 HIV-1 positives and 500 negatives. HIV positivity of 128 plasma samples was confirmed at the Centre for AIDS Reagents (CFAR) using the test**. The samples were then provided to Geneproof to be tested using the PCR Kit*. Clinically HIV negative plasma previously tested for HCV, HIV and HBV negativity by University Hospital Brno were used to set diagnostic specificity of negative samples by PCR Kit*.

Results: The results demonstrated 93% diagnostic sensitivity and 100% diagnostic specificity of PCR Kit*.

Conclusions: The results of clinical performance study demonstrate very good diagnostic parameters of the GP assay. PCR Kit* have proved to be used as convenient diagnostic tool for HIV-1 testing.

*GeneProof HIV type 1 (HIV-1)
* *Cobas Ampliprep/Cobas Taqman HIV-1 Test, v2.0 (Roche)
Aim: HIV antibody immunoassays have a high sensitivity, but frequent false positive tests are also a limiting factor in medical judgement. New test algorithms suggested HIV Ag/Ab assay followed by HIV-1/2 differentiation immunoassay alternative to Western blot (WB). We tried to find out whether additional immunochromatographic assay (ICA) is clinically helpful in a medical setting using 4th generation HIV-1/2 Ag/Ab assays with WB confirmation.

Method: All samples submitted for HIV Ab screening were tested using HIV-1/2 Ag/Ab electrochemiluminescence immunoassay ECLIA; Elecsys HIV combi (PT)*. From Jan 2011 to Dec 2018, 426 sera were positive on ECLIA. All of them were re-tested with an ICA kit; SD Bioline HIV1/2 3.0 ICA** and then requested WB confirmation according to the national policy.

Results: Seventy-one ECLIA positive samples (71/426, 16.7%) showed positive results on ICA and 94.3% (67/71) of them confirmed positive on WB. Four samples (4/71, 5.6%) were false positive on ICA comparing WB. ICA showed negative results in 83.6% (356/426) of ELCIA positive samples and 98.3% of them (350/356) were negative on WB. Five specimens (1.4%) from patients with symptoms compatible to acute HIV syndrome were falsely negative on ICA with positive p24 antigen and indeterminate WB results in initial specimens.

Conclusions: ICA followed by 4th generation HIV immunoassay was helpful for earlier medical decision for frequent false positive screening. But ICA might be falsely negative in patients suspicious of acute HIV syndrome necessitating more sensitive p24 Ag or nucleic acid tests.

*Roche Diagnostic, ** Standard Diagnostics Inc.
EVALUATING THE DETECTION OF HIV-1 RNA AND PROVIRAL DNA IN BUFFY COAT VERSUS WHOLE BLOOD ON ROCHE COBAS AMPLIPREP/COBAS TAQMAN HIV-1 QUALITATIVE TEST

Nur Amelia Natasha Sama¹, Lynette Oon², Wan Loo Tan¹, Kian Sing Chan¹

¹Singapore General Hospital, Singapore, Singapore
²Singapore General Hospital, Pathology, Singapore, Singapore

Aim: "We evaluated the use of enriched buffy coat versus whole blood for the detection of HIV-1 infection on the HIV-1 Qualitative Test*.

Method: "A total of 76 paired non-duplicate clinical samples of whole blood and enriched buffy coat were tested using the modified HIV-1 Qualitative test*. Of these, 41 were known positive samples from patients with previous positive results on the Quantitative assay**, and 35 were samples which tested negative on the HIV-1 Qualitative test*, with concurrent negative HIV serology results.”

Results: “All known positive and negative samples yielded fully concordant results. Of the known positive samples, the use of enriched buffy coat yielded lower Ct values for 38 out of the 41 cases. Two samples gave a lower Ct value with whole blood, and one sample yielded the same Ct value. The lower Ct values obtained with the use of enriched buffy coat versus whole blood were statistically significant (p < 0.05); mean Ct values were 27.78 and 28.99 respectively.”

Conclusions: “This study demonstrated enhanced sensitivity of HIV-1 Qualitative PCR with the use of enriched buffy coat over whole blood for detection of HIV-1. This improvement in sensitivity could be attributed to the higher concentration of infected white blood cells in the buffy coat. We subsequently demonstrated reliable HIV-1 detection from enriched buffy coat in an HIV elite controller, whose whole blood HIV proviral DNA was undetectable.”

* Roche COBAS AmpliPrep/COBAS Taqman (CAP/CTM)
** Roche CAP/CTM HIV-1
Aim: Early diagnosis of HIV in infants (EID) is essential for the timely initiation of antiretroviral therapy in HIV-infected children who experience fast disease progression and high mortality rates. EID is performed by viral nucleic acid test (NAT) which relies on the detection of cell-associated (CA) HIV DNA and HIV RNA. Currently, there are no fit-for-purpose reference materials for the detection of HIV DNA. The goal of this study is to assess the ability of various clinical laboratories to detect CA HIV DNA in order to identify the need for reference materials to help standardize EID.

Method: Six laboratories part of the EPIICAL consortium were provided with a panel of peripheral blood mononuclear cells containing serial dilutions of HIV DNA from subtype A, B and C. The participants were asked to quantify the HIV copy number using their method of choice.

Results: Initial results showed that the detection of HIV DNA from each subtype is highly variable across laboratories and each assay has a different limit of detection. Overall concordance between laboratories varied between subtypes, with subtype B>subtype C>subtype A. The performance of assays improved significantly through protocol optimisation and preliminary data indicate that the use of common materials may help to standardise the detection of HIV DNA.

Conclusions: There is significant variability in the performance of assays detecting CA HIV DNA. This can be reduced by protocol optimisation and the use of common reference materials that allow for effective standardisation.
Aim: The incidence of HPV driven anal cancer (AC) in HIV positive men having sex with men (HIV-MSM) is about 131 per 100000, therefore, a screening for high-grade anal intraepithelial lesions (HGAIN) is justified in that risk population. When using a cut-point of any cytological abnormality, a cytological approach of screening is sensitive for the detection of HGAIN but lacking specificity. An implementation of the HPV test might improve the specificity of cytologically based screening if a proper methodological approach is chosen.

Method: We have tested the performance of HPV genotyping and HPV E6/E7 mRNA expression test in anal liquid-based cytology taken from 174 Czech HIV-MSM. Positivity for any high-risk HPV (hrHPV) type, HPV types 16/18/45, and hrHPV E6/E7 mRNA expression were evaluated in the cytologically positive and negative group.

Results: 95 samples rendered abnormal cytology, 55 were cytologically negative, and 24 were unsatisfactory. In patients with abnormal cytology, 75 (78.9 %) samples tested positive for any hrHPV type, with hrHPV E6/E7 mRNA expression proven in 48 (50.5 %), HPV types 16/18/45 detected in 40 (42.1 %) patients. In the cytologically negative group, 45 (81.8 %) samples tested positive for any hrHPV type, with transcriptional activity proven in 6 (10.9 %), and HPV types 16/18/45 detected in 14 (25.5 %) patients.

Conclusions: Abnormal screening cytology result was associated with the presence of hrHPV E6/E7 mRNA expression (p < 0.05). This indicates a possible utility of hrHPV E6/E7 mRNA expression test in AC screening algorithm.
CLINICAL EVALUATION OF COMMERCIALLY AVAILABLE HIV-1 VIRAL LOAD ASSAYS ACROSS DIFFERENT HIV-1 STRAINS

Jeffrey Wuitschick, Danijela Lucic, Tetsuya Nakasatomi, Harshil Vyas, Shihai Huang

Abbott Molecular Inc, Des Plaines, United States

Aim: Currently there are several fully automated commercially available HIV-1 viral load assays with Alinity m HIV-1 viral load assay being the most recent addition. Limited comparison is available across some of these newly approved assays and platforms. This study evaluated clinical performance of four most commonly and recently approved commercial assays across different HIV-1 strains.

Method: Assay performance was evaluated across clinical samples from group M (subtype A, B, C, D, F, G), group O, CRF01_AE, CRF02_AG, CRF02 and CRF11 HIV-1 strains. Assay correlation was analyzed with following criteria: correlation coefficient, bias, percentage of samples with <0.5 Log Copies/mL difference, and percentage of samples with >0.5 to <1.0 Log Copies/mL difference.

Results: Study results are summarized in below table.

<table>
<thead>
<tr>
<th>N=number of samples</th>
<th>Alinity m - RealTime N=246</th>
<th>Alinity m - cobas 8800 N=118</th>
<th>Alinity m - Aptima N=123</th>
<th>Cobas 8800 - Aptima N=60</th>
<th>RealTime - Aptima N=124</th>
<th>RealTime - cobas 8800 N=117</th>
</tr>
</thead>
<tbody>
<tr>
<td>R²</td>
<td>0.93</td>
<td>0.96</td>
<td>0.93</td>
<td>0.96</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean Bias (Log)</td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.11</td>
<td>-0.03</td>
<td>-0.07</td>
<td>-0.05</td>
</tr>
<tr>
<td>% Samples Different by &lt; 0.5 Log</td>
<td>98.8</td>
<td>99.2</td>
<td>95.0</td>
<td>96.7</td>
<td>98.4</td>
<td>100</td>
</tr>
<tr>
<td>% Samples Different by &gt; 0.5 to &lt;1.0 Log</td>
<td>1.2</td>
<td>0.8</td>
<td>5.0</td>
<td>3.3</td>
<td>1.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusions: Clinical assessment of the newly introduced Alinity m HIV-1 assay demonstrated excellent correlation with other comparator assays across diverse HIV-1 strains. Minimal bias was observed across platforms, supporting no need for re-baselining when Alinity m HIV-1 is integrated in clinical specimen testing.
[P195] EVALUATION OF INTERFRON-X RELEASING ASSAY (IGRA) RESULTS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PATIENTS

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\textbf{Aim:} HIV infected patients have an increased risk of developing active tuberculosis (TB) even when antiretroviral therapy is given. According to World Health Organization 2017 report, the incidence rate of HIV+TB in Turkey is 0.13/10\textsuperscript{5}. We aimed to evaluate IGRA results in HIV infected patients, retrospectively.

\textbf{Method:} Blood samples of 74 HIV infected patients (79.7\%:male, median age:37; 20.3\%:female, median age:36) which were sent to Immunology Laboratory in Akdeniz University Hospital, Antalya, Turkey for QFT\textsuperscript{-}Plus test\textsuperscript{*} between July 2017-March 2019 were analysed. HIV infected patients were screened during their routine check-up which comprises HIV viral load control, CD\textsubscript{4} cell count and clinical evaluation.

\textbf{Results:} QFT-Plus was positive in 8.1\%(6/74) cases, indicating the presence of latent TB infection. Median CD\textsubscript{4} cell count and HIV viral load were 367(SD$\pm$270) cells/$\mu$l and 4.79(SD$\pm$1.53) logcopy/ml, respectively. Patients with a CD4 cell count <200 cells/$\mu$l were 31.08\%(23/74). Only one indeterminate result was obtained with QFT-Plus which corresponded to a patient with a CD4 cell count <200 cells/$\mu$l and a CD8 cell count <600 cells/$\mu$l.

\textbf{Conclusions:} In our study, no cases of active TB was found among QFT-Plus positive results and there was no impact of CD4 cell count on the performance of QFT-Plus test. IGRA is a useful and specific test for diagnosing latent TB in HIV population even if not being in a high endemic region.

\textsuperscript{*}Qiagen, Germany
[P196] PHYLOGENETIC ANALYSIS AND MUTATION PROFILE OF HIV-1 G+CRF02_AG ISOLATES IN BOSNIA AND HERZEGOVINA

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Aim: “To compare phylogenetic analysis and mutation profile of HIV-1 isolates from Bosnia and Herzegovina (B&H) carrying combined subtype G and circulating recombinant form (CRF02_AG), and to identify possible polymorphisms conferring the differences in antiretroviral drug susceptibility.”

Method: “HIV-1 Genotyping System was performed on 52 samples collected from treated and treatment-naïve patients during 2014-2018 in B&H. Stanford HIV Drug Resistance Database (HIVdb) version 8.8 and MEGA5 software were used for data analysis.”

Results: “Two cases of G+CRF02_AG were detected, showing the best match with Saudi Arabia (2003) isolate G+CRF02_AG (CRF43_02G, 3.46% and 3.69%, respectively). In both sequences, the Major and Accessory Resistance Mutations to Protease Inhibitors (PIs) have not been observed, while among Other Mutations of the protease region, K20I and V82I were found. Resistance mutations to Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) have not been detected. Shared Other Mutations to NRTIs/NNRTIs were observed at positions E6D, V35IT, V60I, K64KR, K104R, K122E, D123S, K173T, Q174K, D177E, G196E, T200A, Q207E, V245Q, E248D, D250E, K277R, K281R, T286A, E291D, V292I, I293V, P294T, E297K, S322T, and G335D. Additionally, one sequence revealed T39S and K166T mutations among them, while in the second sequence, K64KR have been detected.”

Conclusions: “This is the first evidence of HIV-1 isolates carrying a recombinant structure composed of subtype G and CRF02_AG, designated as CRF43_02G, susceptible to antiretroviral drugs, in B&H.”
[P197] CLINICAL AND EPIDEMIOLOGICAL IMPACT OF HUMAN PARAINFLUENZA VIRUSES INFECTION IN IMMUNOCOMPETENT AND IMMUNOCOMPROMISED PATIENTS

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**Aim:** Respiratory infections due to human parainfluenza viruses (hPIVs) are widely underestimated in Italy. HPIVs cause also severe syndromes and they circulate in all seasons of the year. The aim of present study was to investigate the epidemiology of hPIVs infections and their clinical manifestation in a hospital-based population.

**Method:** A retrospective study of the circulation of different HPIV genotypes was performed analyzing all respiratory samples positive for HPIVs in the period August 2016 to August 2018. Respiratory samples were routinely tested with a panel of in-house real-time RT-PCR for detection and quantification of respiratory viruses, including HPIVs 1-4.

**Results:** A total of 104 (2.4% of a total) patients resulted positive for hPIVs. Of them, 67/104 (64.5%) had signs and symptoms of URTI, while 37/104 (36.5%) had signs and symptoms of LRTI. The most frequently detected genotype was hPIV-3 (96/104, 92.3%) followed by hPIV-4 (7/104, 6.1%), and hPIV-2 (1/104, 0.9%), while no cases of hPIV-1 were observed. Three outbreaks of HPIV-3 were observed during the study period in onco-hematological ward. Prolonged hPIV shedding (>21 days) was observed in 8 immunocompromised patients.

**Conclusions:** In this study hPIV infection is a considerable factor of morbidity in adults as well as in children. hPIV-3 was the most representative and widespread genotype, with a serious impact as cause of intra-hospital outbreak. The introduction of multiplexed molecular panel for the diagnosis of respiratory viruses have raised the attention on the role of non-influenza viruses as a cause of severe and/or prolonged infections.
Aim: “To assess national data about the requests and results of anti-rubella IgM/IgG analyses in a specific Belgian population.”

Method: “A questionnaire was sent out by the national reference centre (NRC) to 130 Belgian labs, applicable to all anti-rubella IgM/IgG analyses in 2017 in women aged 15-45 years. Data from 109 Belgian laboratories were collected (response rate 84%) about the kit and cutoff used, the number of analyses performed and the qualitative results, the number of referrals and confirmed rubella cases.”

Results: “The used cutoff for IgG assays, calibrated against the World Health Organization International Standard, ranges from 5 to 15 IU/mL. In total 169494 IgG analyses were performed, mainly on 1. (55%), 2. (17%) and 3. (13%) analyzers. Assumed median seroprevalence ranges from 76% with 4. to 96% with Modular*. In contrast to the very low prevalence of rubella disease, 85957 IgM analyses were performed in 2017, with 748 positive and 394 grey zone results. The NRC did not confirm any positive case in 2017 in the 219 referred samples.”

Conclusions: “Retrospective analysis of national data suggests a wide variety in sensitivity and specificity of diagnostic assays measuring anti-rubella IgM and IgG. Large differences in seroprevalence are observed depending on the assay used, despite high vaccination coverage. The number of IgM rubella analyses was very high with no confirmed positive cases, implicating that positive IgM results in women of childbearing age should be interpreted with caution.”

[P199] SEROPREVALENCES OF TEN TORCH INFECTIOUS PATHOGENS IN WOMEN RESIDING IN EUROPE AND LATIN AMERICA

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Aim: “The determination of antibodies against ToRCH antigens before or at the beginning of pregnancy allows both assessment of the maternal immune status and the risks to an adverse pregnancy outcome. Here, we compared the seroprevalences of ten ToRCH infectious agents in women of childbearing age between five countries.”

Method: “Seroprevalences were determined in sera of healthy women (N=752, 15-46 years) residing in Germany (n=202), Turkey (n=97), Mexico (n=100), Brazil (n=160) or Poland (n=193) using the EUROLINE Anti-TO.R.C.H. 10-Profile (IgG)*. This lineblot contains native and recombinant antigen substrates against five standard (Toxoplasma gondii, rubella virus, cytomegalovirus (CMV), herpes simplex viruses (HSV-1, HSV-2)), and five additional (Bordetella pertussis, Chlamydia trachomatis, parvovirus B19, varicella zoster virus (VZV) and Treponema pallidum) pregnancy-related pathogens. The EURLineScan software* was used to evaluate band intensities.”

Results: “Seroprevalences of rubella virus (seroprevalence range across five countries: 94-99%), VZV (range: 94-99%), parvovirus B19 (range: 36-55%) and Treponema pallidum (range: 1-3%) antibodies were comparable. Across countries, Brazil showed significantly elevated seroprevalences for HSV-1 (range: 74-95%), HSV-2 (range: 2-39%), Toxoplasma gondii (range: 24-59%), Chlamydia trachomatis (range: 5-45%) and Bordetella pertussis (range: 1-59%) antibodies. German women had a significantly lower seroprevalence of anti-CMV antibodies (range: 38-99%).”

Conclusions: “The observed differences in seroprevalences advocate country-specific infection prophylaxis strategies and close monitoring as part of prenatal care. By incorporating antigens against five additional, pregnancy-related infectious agents, the EUROLINE Anti-TO.R.C.H. 10-Profile (IgG) enables an extensive and superior assessment of maternal immunity and consequent risks for an adverse pregnancy outcome.”

*Euroimmun AG, Germany
Herpes simplex viruses consist of two strains known as HSV-1 and HSV-2. Studies have shown higher prevalence of HSV-2 in females and positive effect of progesterone on HSV-2 infection in vitro.

**Aim:** Examine HSV-1/2 infection/ reactivation characteristics, such as distribution between gender and comorbidity.

**Method:** Our data was obtained from the Israeli national center of herpes viruses where genital swabs are routinely tested for HSV-1 and HSV-2 infection/ reactivation by automated DNA extraction followed by multiplex real-time PCR.

**Results:** From 2011-2017, we diagnosed 6952 samples, 1548 from men and 5404 from women. Higher prevalence of HSV-2 was observed in females compared to males (11.36% compared to 4.39% respectively, p<0.01). By screening medical records of hospitalized women positive for either HSV-1 or HSV-2, we found that while comorbidity with different malignancies did not differ among the two strains, HSV-2 was more prevalent in pregnancy than HSV-1 (38.46% compared to 16.89% respectively, p<0.01).

**Conclusions:** The results indicate that pregnancy might play a role in HSV-2 infections or reactivations. It may also partially explain the difference in HSV-2 prevalence between genders. Our data suggests that pregnant women latently infected with HSV-2 should be aware of a possible reactivation episode during pregnancy.
[P201] COMPARISON OF LIAISON VZV IGG AND SERION CLASSIC VZV IGG. WHICH CUT-OFF FOR IMMUNITY SHOULD BE USED?

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Aim: Knowledge of the varicella-zoster virus (VZV) immune status of health care workers, patients before immunosuppressive therapy and woman prior/during pregnancy is required to identify individuals who need vaccination or VZV immune globulin prophylaxis following exposure to VZV. VZV IgG and SERION ELISA classic Varicella-Zoster Virus IgG (Serion) are two widely used, commercially available VZV-IgG immunoassays to quantitatively determine VZV-IgG in serum. Compared to Serion, Liaison is fully automated, has a shorter turnaround time and allows continuous sample loading. For quantitative immune-assays Rober-Koch-Institutes recommends a positivity cut-off of 100 IU/ml. However, Liaison offers two cut-off values: 100IU/L or 165IU/L. Here we compare the back-to-back performance of Liaison and Serion for quantification of VZV-IgG to determine the cut-off value for positivity when switching from Serion to Liaison.

Method: For total of 135 samples, VZV-IgG were determined using Serion and Liaison. Sensitivity and specificity of Liaison (compared to Serion) using both cut-offs were calculated. Results were analyzed by Passing-Bablok analysis, Bland-Altman plotting and Spearman correlation analysis.

Results: Liaison attained a sensitivity of 97% and specificities of 77.6% (100IU/mL) and 91% (165IU/mL), respectively. Despite acceptable overall correlation (Spearman’s Rho: R=0.927), Passing-Bablok analysis (slope 1.64) and Bland-Altman Plot (mean of differences Liaison/Serion: 235.3 IU/L) revealed that Liaison measured systematically higher IU values of VZV-IgG when compared to Serion.

Conclusions: Because of the systematically higher quantification of VZV-IgG by Liaison compared to Serion, we suggest a positivity cut-off of 165IU/L for Liaison when switching between these two systems.

* DiaSorin LIAISON®
ASSOCIATION OF TAP2 GEN POLYMORPHISMS WITH RECURRENT RESPIRATORY PAPILLOMATOSIS IN PATIENTS FROM WESTERN MEXICO

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Aim: “The aim of this study was to examine a polymorphism in TAP2 gen, involved in antigen presentation by antigenic peptides transport process to the rough endoplasmic reticulum (RER), and determine the association between TAP2 (T665A) with recurrent respiratory papillomatosis (RRP).”

Method: “A case-control study was carried out in a group of 80 individuals (40 healthy unrelated individuals and 40 patients diagnosed with RRP) from two public hospitals in western Mexico. Genomic DNA from whole blood was extracted by standard techniques using High Pure PCR Template Preparation Kit and the identification of polymorphism in TAP2 through allelic discrimination employing TaqMan probes by qPCR were performed. The analysis of genotypic, allelic frequencies and odds ratio (OR) calculations were estimated with OpenEpi.com Statcalc using a p-value of 0.05.”

Results: “Samples were collected from 40 patients with RRP from 2014 to 2018; with an age range 2-73 years. Significant differences were found, the polymorphic allele was found in a greater proportion in the group of RRP patients (p= <0.05) vs healthy individuals. An OR = 4.38, 95% CI (2.03-9.43) p = <0.05 was obtained.”

Conclusions: “This is the first study to demonstrate that TAP2 polymorphism T665A is a risk factor associated with RRP in Mexican population.”
Enterovirus Surveillance of 2018 Reveals an Upsurge of EV-D68 in Belgium

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Aim: “Enteroviruses (EVs) are responsible for a wide range of clinical symptoms. EV-D68 was already known to cause mild to severe respiratory infections, but in the last few years, it has also been associated with neurological symptoms and acute flaccid paralysis (AFP), a polio-like disorder. In this Belgian study, EVs circulating in 2018 were molecularly typed in different sample types.”

Method: “EV-positive samples were collected from the University Hospitals Leuven and other hospitals and medical practices in Belgium from January to December 2018. Samples included cerebrospinal fluids (CFS), respiratory samples, feces and skin lesions. Molecular typing was done by RT-PCR using multiple primers sets, followed by sequencing part of the VP1 gene. EV-D68 was typed in the VP4/VP2 region and phylogenetic analysis was performed.”

Results: “Twenty-five different EV genotypes were detected and the five most identified EVs were EV-D68 (15.1%), E-30 (13.3%), E-11 (12.7%), E-9 (12.0%) and E-6 (6.6%). One case of the sporadically reported EV-C104 was detected. The first outbreak of EV-D68 in Belgium occurred in 2018, associated with severe respiratory infections but no AFP. Phylogenetic analysis showed that the outbreak samples coexist in different clades and subclades.”

Conclusions: “For epidemiological surveillance, 166 EV-positive samples were molecularly typed. 25 different genotypes were detected in one year. The data revealed an upsurge of EV-D68 in respiratory samples and E-30 was predominant in CSF. This report demonstrates that EV-D68 is not always associated with serious symptoms.”
BRAZILIAN VIRAL HEMORRHAGIC FEVERS: YELLOW FEVER AND HANTAVIRUS CARDIOPULMONARY SYNDROME

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Aim: I show here 2 severe hemorrhagic fevers with different clinical presentations are described here

Method:
1. Yellow fever (YF), a Flavivirus disease transmitted by mosquito bite and carried by primates. In the 2015-19 Southeastern Brazilian unexpected outbreak of YF, 2187 cases and 745 deaths were reported. In order to control outbreak, Brazilians have been fastly immunized with YF17DD attenuated virus vaccine.
2. Hantavirus Cardiopulmonary Syndrome (HCPS) is caused by Orthohantavirus Araraquara (ARQV), transmitted by inhalation of infected wild rodents’ excreta. There were 63 reported cases of HCPS in 2017 and 35 in 2018.

Results:
1- Based on clinical and necropsy findings of 2 unvaccinated rural men, both started with acute febrile illness, evolving to fulminant hepatitis, shock and death in a couple of days.
2- Clinical outcome of this severe disease, based on experience of 70 cases, resulted in 50% lethality. After 2 days of inespecific acute febrile illness, patients evolved to respiratory failure and shock. HCPS was produced by a cytokine storm which induced exacerbated inflammatory response, leading to myocarditis and capillary leaking of plasma to lung interstitium. HCPS was more frequent in TNF-308 hyperproducer allele carriers.

Conclusions:
1. New approaches for treatment of YF include prescription of oral sofosbuvir and liver transplantation;
2. Poor prognosis of HCPS associated to unsuspected diagnosis at hospital admission, infusion of more than 2000ml of parenteral fluid in the first 24 hours of hospitalization. Precocious assistance of ventilation and inotropic drugs infusion were critical for patient’s survival.
[P205] VIRAL HAEMORRHAGIC FEVER READINESS: MORE THAN AN ALGORITHM?

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Aim: Viral haemorrhagic fever (VHF) is classed as a high consequence infection. The mismanagement of a suspected VHF case could result in a life-threatening transmission. In the past decade, inclusive of the West African outbreak, northern Ireland saw 6 patients presenting with suspected VHF. We feel simulation based training can offer a safe learning environment for staff members to gain necessary skills. We present an exercise in simulation looking at the management of a suspected VHF case in Belfast, Northern Ireland.

Method: A simulated patient presented to the A&E department. The patient was assessed by A&E staff and transferred to the intermediate isolation unit under the care of the Infectious diseases team. Blood samples were drawn and processed by the laboratory, along with a sample couriered to the PHE Rare and Imported Pathogens Laboratory in England for VHF screening. The process of triage and employment of appropriate algorithms were assessed by observers.

Results: The exercise highlighted areas that the team carried out well, but also revealed aspects where the policy did not work well. Formal feedback sessions were running to discuss and implement change were required.

Conclusions: As higher specialist trainees the process of organising and implementing a simulation of this nature allowed us to develop leadership roles. All participants felt the simulation identified issues that could not have been uncovered by any other means. We conclude that policy making and training in infections of high consequence, but low incidence is best served by the implementation of regular simulation.