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Abstracts of the 19th Annual Meeting of the European Society for Clinical Virology
14th–17th September 2016, Lisbon

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JOURNAL OF CLINICAL VIROLOGY

Aims and Scope

The Journal of Clinical Virology (JCV) is an international journal publishing papers on any aspect of human virology that directly pertains to virus-induced clinical conditions. Articles from any field of virological study will be considered if the article is relevant to the understanding or manipulation of a disease state.

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Abstracts of the 19th Annual Meeting of the European Society for Clinical Virology, 14th–17th September 2016, Lisbon

Abstract no: 75
Presentation at ESCV 2016: Oral 1

Medico-economic impact of the rapid diagnosis of influenza in paediatric emergency department by using a new sensitive chromatographic immunoassay

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Background: The practicability of rapid immunochromatographic antigen detection tests (RIADT) for the diagnosis of influenza allows their use by non-trained healthcare professionals, even beside the bed of the patient. Newly designed assays such as the Becton Dickinson (BD) Veritor™ System for Rapid Detection of Flu A + B have shown improved analytical performances. However, the medico-economic impact of their direct implementation in clinical wards has been poorly evaluated.

Objectives: (1) To measure the impact of the rapid diagnosis of influenza by using the Veritor™ System directly in the paediatric emergency room, on the care of patients and notably on the reduction of supplementary investigations and antibiotics during the 2015–2016 winter season, and (2) to verify the analytical performances of this new test.

Study design: A nasopharyngeal aspirate was performed to all the patients aged between 0 and 18 years consulting at the paediatric emergency department of the University Hospital of Saint-Etienne during the 2016 influenza season. The presence of influenza A and B viruses was tested with the Veritor™ System in the emergency room 24/7. The clinical specimen was also sent to the laboratory in order to perform routine tests in the opening hours, including the detection of viral antigens by immunofluorescence (bioMérieux) or of viral RNA by using a laboratory-developed RT-qPCR assay on the BD MAX platform. In case of discrepant result between the different assays, the final diagnosis was assessed by the RT-qPCR assay. Before performing the Veritor™ System, the clinician was asked to fill in a questionnaire listing the tests that he/she would have prescribed in the absence of the rapid testing; the same questionnaire was completed after the result of the Veritor™ test was available.

Results: A total of 514 patients (sex ratio M/F: 1.11; median of age 2.7 years) were included between January 7 and March 19 2016. Comparative results between the RIADT and the routine assays were available for 477 specimens, including 45 and 185 positive ones for influenza A and influenza B, respectively. For influenza virus A, the sensitivity, specificity, negative predictive value and positive predictive value were 94.9, 98.2, 96.4 and 97.4%, respectively; for influenza virus B, they were 97.4, 96.4, 98.2 and 94.9% respectively. The use of the RIADT at the emergency room saved the prescription of: 51.7% of C-reactive protein (CRP) dosage in capillary blood, 47.9% of blood sampling, 49.2% of CRP measurement, 57.4% of procalcitonin dosage, 68.8% of blood culture, 41.6% of urine test strip, 79.2% of cytobacteriological examination of urine, 69.0% of chest X-rays, 77.8% of lumbar puncture, 43.8% of hospitalization and 70.1% of antibiotics.

Conclusions: The excellent analytical performances of the Veritor™ System allow its use as point-of-care test to perform an accurate and rapid diagnosis of influenza at the paediatric emergency room. In addition to deliver a rapid etiological diagnosis, this strategy was found to save the prescription of a considerable amount of unnecessary tests and antibiotics.

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Abstract no: 306
Presentation at ESCV 2016: Oral 2

Molecular characterization of human rhinovirus in Portugal: 2014–2015 season

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Background: Human rhinoviruses (HRV) frequently cause mild upper respiratory tract infections and more severe disease manifestations such as bronchiolitis and pneumonia. Plays an important role in asthma and chronic lung disease exacerbations. Genetic characterization of HRVs detected by molecular methods has revealed much greater diversity, enabling the identification of three species (HRV-A, HRV-B and HRV-C) and a great number of types. The aim of this study is to characterize HRV detected in respiratory specimens from influenza-like illness cases (ILI), received in

the scope of the Portuguese Influenza Surveillance Program, during 2014–2015 season in Portugal.

Methods: During the 2014–2015 season, 411 nasopharyngeal swabs negative for influenza were tested for HRV in a multiplex PCR [1]. 18% (75/411) of the samples were positive for HRV, and from this 83% (62) were sequenced by a nested RT-PCR [2]. Nucleotide sequences of the VP4/VP2 region were used for genotyping and phylogenetic tree construction in Mega 6.0. Demographic and clinical data (according to EU ILI case definition) were recorded in a questionnaire.

Results: HRV were detected throughout the study period, between week 40/2014 (October) and week 9/2015 (March) with a peak in January 2015. Phylogenetic analysis showed that 45% (28/62) strains belonged to species HRV-A, 15% (9/62) to species B and 40% (25/62) to species C. Overall were identified 35 different types. All species co-circulated in Portugal with the exception of the Algarve and Açores, being HRV-A predominant in north region and HRV-C predominant in Alentejo. HRV positives cases had a median age of 42.5. HRV-C were the most frequently detected in all age groups, apart from young adults aged 15 to 44. In this age group HRV-A were identified in 73% (16/22). HRV-B was detected sporadically in all age groups, except in children (5-14 years old). HRV was found in similar proportions in both genders (52% in female; 48% in male). Data on influenza vaccination was reported in 51 HRV positive cases, but only 9 (18%) had been previously vaccinated. Information on chronic diseases was reported in 54 cases, of these 15 (28%) had a chronic disease (mainly cardiovascular or diabetes). HRV was detected in 4 pregnant women, 14% (4/28). Cough, myalgias, weakness and fever were the most frequent symptoms reported by HRV confirmed cases.

Conclusions: During 2014–2015 was observed a co-circulation of the three species of HRV (A, B and C) with a predominance of HRV-A followed by the recently identified species C. A wide genetic diversity of 35 types was identified, with a higher diversity among HRV-A. HRV was most frequently diagnosed in adults. Our study included few children under 5, preventing conclusions about this group. Diabetes and cardiovascular disease were found as a possible risk for HRV infection, highlighting the relevance of respiratory disease prevention measures that should be undertaken. This was the first study to attempt the genetic diversity of rhinovirus circulating in Portugal during a winter season in ILI cases. Further studies in the general population and in high-risk groups for severe respiratory disease will aid knowledge in HRV epidemiology and exacerbation of respiratory infections.

References

[1] R.N. Gunson, et al., J. Clin. Virol. 33 (2005) 341–344.
[2] P. Linsuwanon, et al., J. Infect. 59 (2009) 115–121.

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Abstract no: 224
Presentation at ESCV 2016: Oral 3

Illuminating influenza epidemiology in Scotland using next generation sequencing

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Influenza is one of the most important respiratory pathogens and is a major cause of mortality and morbidity worldwide every year. Influenza A is a n RNA virus consisting of eight segments, the segmented nature of the genome allows for reassortment to occur, occasionally producing antigenically novel viruses capable of causing influenza pandemics. The ECDC Scottish influenza laboratory currently characterise influenza A isolates by sequencing the HA1 region of the haemagglutinin gene, which allows isolates to be classified into viral clades.

One hundred and fifty clinical isolates positive for influenza A(H3N2) from the 2014/15 influenza season were sequenced by both Sanger sequencing of HA1, in addition to whole genome sequencing using next generation sequencing (NGS) technology on the Illumina MiSeq platform. Influenza nucleic acid was amplified using a single-reaction method, which simultaneously amplifies all eight segments of the influenza genome. This amplified product was then utilised for NGS.

Full segment coverage was achieved for the smaller segments (NS and MP) of all 150 isolates, however coverage generally decreased as the size of the segment increased. In total, 100% genome coverage was achieved in 71 samples, with 100 samples having >90% genome coverage. Sequencing of the haemagglutinin gene was adequate for clade calling for all 150 isolates and phylogenies of the haemagglutinin gene constructed using NGS data had better resolution than those produced using Sanger sequencing of HA1 alone. In addition, using whole genome data we were able to analyse isolates for evidence of viral reassortment and identified a number of intra-clade reassortments in our dataset, involving both the surface glycoproteins and internal genes. The majority of these occurred sporadically, however one reassortant virus persisted in the population.

Current routine influenza surveillance relying on sequencing of the HA1 region allows for classification of influenza A viruses into viral clades. Whole genome sequence data produced using a single-reaction method and NGS allows for economical generation of viral clade classification in addition to sequence data from the other seven segments. In our small dataset we identified a number of viral reassortments, suggesting that such events may occur more often than previously estimated.

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Abstract no: 195
Presentation at ESCV 2016: Oral 4

Long-term impairment attributable to congenital cytomegalovirus infection

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Introduction: Congenital cytomegalovirus infection (cCMV) is the most prevalent congenital infection worldwide and it may lead to symptoms at birth as well as long term sequelae. Limited data on long term sequelae are available, particularly in infants who are asymptomatic at birth and in many studies on long-term consequences a control group is lacking.

Aim and methods: A nation-wide retrospective cohort study was designed to assess the long term consequences of cCMV up to

the age of six years in the Netherlands. cCMV was diagnosed using polymerase chain reaction on 31,484 stored dried blood spots, collected for neonatal screening purposes. Medical data on 133 cCMV-positive children and 274 cCMV-negative controls (matched for age, gender and region), were obtained from general physicians and other health care providers. Symptoms in the neonatal period and moderate to severe long-term impairments, in hearing, visual, neurological, motor, cognitive and speech-language, were analyzed.

Results: In the cCMV-positive group 26 (19.6%) children were classified symptomatic at birth, whereas in the cCMV negative group 34 (12.4%) children had similar symptoms. Overall 33 (24.8%) cCMV-positive and 33 (12.0%) cCMV-negative children had one or more long term impairments (risk difference: 12.8%, 95% CI: 4.5–21.1). Long-term impairment was more common in children with cCMV who were symptomatic at birth (53.9%) compared to cCMV-positive children without symptoms at birth (17.8%).

Sensorineural hearing loss was only seen in children with cCMV (3.8%). Impairments in cognitive, motor and speech-language development were respectively 5.5, 7.2 and 2.2 times more frequent in children with cCMV compared to the control group without cCMV. Impairment in multiple domains was also more common in children with cCMV (10.5%), especially in children with symptoms at birth (19.2%), compared to children without cCMV (1.8%).

Conclusion: Long term impairments were more common in the cCMV positive than the cCMV negative children. Especially hearing loss, cognitive, motor and speech-language impairment is more common in children with cCMV. Children with cCMV who are symptomatic at birth have an even higher risk of one or multiple impairments compared to asymptomatic cCMV-positive children and children without cCMV.

These findings demonstrate the need to reinterpret the role that congenital CMV infection plays in causing symptoms at birth as well as long term sequelae.

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Abstract no: 32
Presentation at ESCV 2016: Oral 5

Severe fetopathy caused by cytomegalovirus recurrence with isolated intra-abdominal complication in an immune woman

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Introduction: Cytomegalovirus (CMV) is the main cause of foetal infection: 1% of neonates are CMV-infected and among these, 10% are symptomatic at birth. Despite this prevalence, only anecdotal cases of severe congenital CMV disease have been reported. We report here the case of a pregnant woman with CMV recurrence linked to an atypical clinical presentation, leading to the abortion of her first pregnancy.

Case report: A healthy 23 years-old woman (gravida 1, para 0) became pregnant without any clinical or biological problem during the first trimester. A systematic foetal ultrasound, at 22 weeks of gestation, showed a large intra-abdominal hyper echogenic and heterogenic cystic mass. These results were confirmed by foetal MRI and meconial peritonitis was suspected. Extra- and intra-abdominal complications were explored and a cardio- and a splenomegaly were diagnosed without any evolution during all the pregnancy. There was no neurological signs, no variation of the amniotic fluid volume and no cardiologic dysfunction. The maternal serological assay done before and during pregnancy showed constant low levels of IgM anti-CMV (between 0.5 and 0.7 IU/ml) and high level of IgG anti-CMV (between 245 and 402 IU/ml) (Beckman-Coulter, USA; Abbott, USA). Inflammatory markers (CRP, fibrinogen, neutrophils cells) were all up-regulated but there was no thrombocytemia in foetal and maternal blood. Foetal amniocentesis showed a high CMV viral load in amniotic fluid (7.5 log copies/ml) and foetal blood (6.1 log copies/ml) (bioMérieux, France). At the same time, CMV viral load was slightly positive in the mother's blood (198 copies/ml) (Vela diagnostics, Germany). Sequencing of CMV strains is currently ongoing in order to explore possible viral severity markers. All the other fetopathy causes were negatives (genetic: trisomy 21, cystic fibrosis/metabolic diseases/immunologic: Kleihauer test/infectious: Parvovirus B19). The pregnancy was interrupted at 34 weeks, because of the major risk of intestinal sequelae, leading to foetal death. There was no post-mortem examination of the corpse due to the parents' opposition. However, pathological examination of the placenta revealed hydrophic chorionic villi with cytomegalic inclusion bodies and positive anti-CMV nuclear staining (Ventana, Argene, clone E13).

Discussion: We showed here a case of CMV recurrence leading to severe complications of the foetal development, in a pregnant woman considered to be immune. The absence of neurological (microcephaly and periventricular calcifications) or hematologic (thrombocytemic purpura) signs caused a delayed diagnosis. The isolated intra-abdominal abnormalities were an atypical but severe presentation of CMV's infection of the foetus. In conclusion, our case-report highlighted the need for an optimized partnership between hospital clinicians and private biologist. A systematic viral screening of foetal infections (*Parvoviridae* or *Herpesviridae*) should be proposed in front of any abnormalities during the foetal ultrasound examination. The use of symptom multiplex screening assays on amniotic fluid may help in these difficult situations to optimize management of patients in order to detect or prevent fatal outcomes.

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Abstract no: 349
Presentation at ESCV 2016: Oral 6

Current trends in molecular epidemiology of Varicella-Zoster Virus clinical isolates in Czech republic

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Infections with Varicella-Zoster Virus (VZV) in humans manifest by two different sets of symptoms – chickenpox and shingles. These infections exhibit annual peaks, as well as geographical distributions of different virus genotypes and predilection of each of the 2 manifestations in defined age groups.

During the period 2009–2016 we followed a total of 898 patients with either chickenpox or shingles. A complete analysis of anamnestic data was performed and statistically evaluated. Furthermore, in 567 virus isolates from these patients, genotypization of VZV was performed.

Results confirmed our previous findings (on a smaller cohort) and showed a higher prevalence of VZV E2 genotype (compared to the E1strain), contrary to the reports from western Europe. In the patients with chickenpox the E1 strain was found in 28,3% of isolates, while the E2 strain in 70,5% of isolates. In patients with shingles/herpes zoster the prevalences of the individual strains were 33.3% of E1 and 66.1% of E2. In general, the E2 strains were more prevalent in the Czech population with 385 positive patients out out of the 567 total (67.9%). As part of this typization study several VZV strains with newly DNA polymorphisms were described.

In addition, we have performed Next-Generation-Sequencing (NGS) analysis in 13 VZV isolates. This led to the identification of an European strain with two new mutations in an immunosuppressed child. Several of these strains were also analyzed for their virulence by the real-time monitoring of the infection in cell culture using the xCELLigence system.

Taken together we present molecular epidemiology data on a large cohort of VZV positive patients and their correlation with both anamnestic data and disease characteristics. In this study, new variants of the VZV strains were found and several genotypes were correlated to the virulence of the individual strains.

Molecular epidemiology is a useful tool for both epidemiological analyses of the individual VZV strain and correlations between the severity of the disease and individual variants.

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Abstract no: 174
Presentation at ESCV 2016: Oral 7

Increased detection of enterovirus type D68 associated with acute flaccid paralysis and severe respiratory illness in Wales, January–February 2016

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A case of acute flaccid paralysis in a two year old child was notified to Public Health Wales in December 2015. Enterovirus RNA was subsequently detected in a faeces sample by in-house RT-PCR. Investigations for poliovirus were negative and enterovirus type D68 (EV-D68) was detected by specific RT-PCR.

Retrospective analysis of respiratory samples collected from children presenting with severe disease during December 2015 were screened by generic enterovirus RT-PCR, this was then followed by testing all those found enterovirus positive and all CSF samples testing enterovirus positive for EV-D68. Two more cases were identified during this time period, both presenting with severe respiratory illness.

Prospective testing was then undertaken of all respiratory and CSF samples submitted for respiratory virus detection from across Wales following the same protocols during January and February 2016. In total, 112 samples were found to be enterovirus positive, 56 of which were positive for EV-D68. As previously described, EV-D68 was not found in any CSF samples but was readily detected in respiratory samples during the acute phase of illness. Further genetic characterisation of the enteroviruses (EV-D68 and non EV-D68) detected as part of this work is ongoing.

Although EV-D68 was found in all age groups, children under the age of 5 years were disproportionally affected, in-keeping with the descriptions from the US outbreak in 2013. Further associated neurological complications were described for two children, including development of a squint in one child and limb weakness in another. However, respiratory complications were the main presenting features with 8 children and one adult requiring intensive care therapy.

With the eradication of poliovirus imminent, it has been well documented that increased surveillance of polio and non-poliovirus enteroviruses should be undertaken importantly, this EV-D68 epidemic in Wales highlights the limitations in current strategies that rely cases of neurological disease and detections in CSF. Since 2014, Public Health Wales routinely tests all respiratory samples submitted through the influenza surveillance scheme from primary care for enteroviruses. One sample from the surveillance scheme tested positive for EV-D68 at the beginning of February 2016 when the number of cases detected in hospital patients was at its peak. We now plan to routinely test all respiratory samples for enteroviruses as part of the routine respiratory panel with the aim to improve detection rates of all enteroviruses and especially those not readily detectable in CSF such as EV-D68.

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Abstract no: 180
Presentation at ESCV 2016: Oral 8

Does a chill cause a cold?

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The common cold is one of the commonest upper respiratory tract infections (URTI) in the world. URTI are caused by a wide range of infectious agents, and often precede more serious lower respiratory tract infections (LRTI). URTI have a seasonal pattern, occurring more frequently during the colder months, with peaks in activity at the start of autumn and spring.

The study aimed to examine the seasonality of the causative agents of URTI and the effects of meteorological factors on this seasonal variation. Meteorological data for the Edinburgh Gogarbank area was acquired for five variables: temperature, pressure, relative humidity, wind speed and dew point. Results of respiratory samples tested at the Royal Infirmary of Edinburgh between 2009 and 2015 were analysed. The agents identified were rhinovirus, adenovirus, influenza viruses A and B, parainfluenza viruses (HPIV) 1, 2 and 3, respiratory syncytial virus (RSV), human metapneumovirus (HMPV) and *Mycoplasma pneumoniae*.

Statistical tests were performed with SPSS to assess the relationship between the seasonal variation of these agents and the meteorological factors. These statistical tests included *t*-tests, ANOVA and post-hoc analysis and the creation of generalised linear models.

Seasonal patterns were identified for each agent by graphical representation with meteorological seasonality. *T*-test and

ANOVA were used to determine the significance of the relationships between agent incidence and the meteorological factors. Significant relationships with temperature, dew point, relative humidity and fluctuation in humidity (humidity-range) were found in many of the infectious agents, with HPIV-3, RSV and Influenza viruses A and B showing the strongest correlations. Influenza viruses A, B and RSV preferred a low temperature, dew point and humidity-range, whilst also preferring a high humidity level. Notably, HPIV-3 showed the opposite relationship. This is the first time, an association between fluctuation in humidity and viral incidence has been described.

A generalised linear model was constructed for each agent to establish a statistically rigorous representation of its seasonal pattern and the relationship with temperature, allowing for the seasonality of all the agents to be confirmed.

The identification of each agent's seasonal peaks allowed for the prediction of the order in which each agent will arise in a given year, starting with HPIV-3 in April, followed by rhinovirus, adenovirus, HPIV-1, HPIV-2, *M. pneumoniae*, RSV, Influenza virus A, Influenza virus B and ending with HMPV in March of the following year. A change either in temperature or humidity or both was associated with transition from one agent to another through the year.

Meteorological factors like temperature and humidity have a significant effect on the incidence of the causative agents of the common cold. This information could now possibly be used to predict the transition from one virus to another. By tracking changes in meteorological factors, medical professionals could now be forewarned of an oncoming rise in infections from a particular agent, allowing them to take appropriate preventative measurers.

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Abstract no: 285
Presentation at ESCV 2016: Oral 9

Metagenomic analysis of the respiratory virome associated with acute respiratory illness of unknown etiology in infants

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Introduction: Acute respiratory infections (ARIs) are the leading cause of illness and death in children under five years old, who experience three to six episodes per year. Viral infections are the main etiology of ARIs but etiologic agents are often not identified. Recent studies have suggested that virome has important effects on human health. Therefore, respiratory virome characterization may help to understand these ARIs of unknown etiology. The aim of the study is to characterize the respiratory virome of children under five with an ARI of undiagnosed etiology in order to identify potential novel respiratory viruses or variants not detected by routine tests. For this purpose, a method for metagenomic analysis of respiratory virome was optimized by implementing a quality control to validate each steps of the process.

Methods: A retrospective study was conducted on samples received at the virology laboratory of the University Hospital of Lyon, France, between 2010 and 2015. Upper respiratory samples from children under five were first selected by an epidemiological approach based on respiratory viruses circulation patterns. As an undiagnosed pathogen could be suspected in numerous infectious diseases with negative biological investigation, "undiagnosed events", defined as periods with proportion of negative samples > 75% of the total number of samples received in the laboratory, University Hospital of Lyon, were targeted. Samples with a high probability of viral infections were selected according to the following criteria: absence of documented infection with routine techniques used at the time of diagnostic on at least 2 negative respiratory samples collected within 14 days of their admission (hospitalization time > 24 h). These samples were then controlled by a sensitive multiplex nested Polymerase Chain Reaction (FilmArray® Respiratory Panel (FA RP), bioMérieux, Lyon, France). After exclusion of positive samples with this technique, a quality control (viral strain) was added to each sample before metagenomic analysis.

Results: 223 patients were identified by targeting "undiagnosed events" as described. Twenty-two patients with high probability of viral infections were selected, 13/22 (59.1%) were under one years old and 14/22 patients (63.6%) had comorbidities (mainly respiratory chronic diseases). Patients developed mostly signs of upper respiratory tract infection, such as cough and rhinorrhea, but two patients developed severe respiratory distress with the need of ventilation. Viruses were detected in most of the samples with FA RP (17/22) (77.3%) mainly *Picornaviridae* viruses (13/22) (59.1%). A metagenomic analysis with a quality control process was developed. An optimized metagenomic protocol was successfully used for five negative patients. Sequencing analysis are currently in progress.

Conclusion: No prior studies performed metagenomics analysis to characterize the respiratory virome involved in ARIs of unknown etiology in infants. Identification of a novel respiratory virus could have a strong impact on ARIs diagnosis and management. In absence of new virus identification, this study has produced useful results describing the respiratory virome of children with ARIs. Characterization of the whole viral communities detectable in the human respiratory tract is key for understanding the role of the virome in respiratory disease.

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Abstract no: 37
Presentation at ESCV 2016: Oral 10

Assumption-free improvement of the *maxRatio* algorithm increases the accuracy of qPCR assays targeting viruses

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Introduction: Quantitative PCR (qPCR) is widely applied in Laboratories of Virology worldwide for screening, diagnostic and research purposes. Analysis of qPCR data is typically performed with the cycle-threshold method (CT), which requires the assignment of the cut-off and baseline range by the operator. These assumptions might impair the reproducibility of the results between laboratories and could underestimate the impact of inhibition of the reaction of amplification. The *maxRatio* method

(MR) was introduced in order to analyse the qPCR data without input from the operator, accounting at the same time for sub-optimal reactions. In the present work, we modified MR to filter out results inconsistent with positive reactions using an assumption-free approach. We applied this novel algorithm of qPCR analysis to several primer sets targeting a plethora of viruses in order to assess its effectiveness with respect to the CT.

Methods: Clinical samples ($n = 328$) were obtained from residual faecal specimens processed by the *Clinical Microbiology and Public Health Laboratory* at Addenbrooke's Hospital (Cambridge, UK). The samples were extracted by *QIAsymphony SP* and amplified on *Custom TaqMan Array 384-well Card* by *TaqMan Fast Virus 1-Step Master Mix 2×* on *Viia7* thermalcyclers. The results were issued as either positive or negative by three operators and a consensus classification was generated. A training dataset of 1920 reactions was obtained from a pool of 54 primer sets performed over 50 plates. The resulting MR data were analysed by EM algorithm to obtain a cut-off for the positive/negative results. This filtered MR was then applied to 23 primer sets targeting different viruses for a total of 6038 reactions. MR values below the empirical cut-off were considered negative and the consensus classification was used to assess the accuracy of detection in comparison to CT.

Results: Five of the 23 primer set analysed (21.74%) showed a better accuracy and negative predictive value using the MR rather than the CT method, both being in average 0.987 ± 0.013 and 0.996 ± 0.006 for the CT and MR, respectively. The clinical sensitivity for four of these primer sets was in average 0.500 ± 0.136 and 0.764 ± 0.274 for the CT and MR, respectively; for the single primer set where this parameter could not be computed, CT and MR showed ten and none false negative reactions, respectively. In all other instances, CT and MR performed equally.

Discussion: The data gathered suggested that MR is a competitive analytical algorithm for qPCR analysis, providing higher accuracy than CT in one fifth of the targets tested while being comparable to CT in all other cases. MR also had the advantage over CT of (a) being assumption-free and (b) taking into account primer specific inhibitions. The use of MR can be beneficial for several qPCR applications by increasing the effectiveness and reproducibility of the assay. MR can also assist the operators during the visual inspection of the individual reactions by highlighting problems in the amplification.

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Abstract no: 202
Presentation at ESCV 2016: Oral 11

Anti-BK virus neutralizing antibody titers before transplantation predict BK virus replication in kidney transplant recipients after transplantation

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BK virus-associated nephropathy (BKVN) is the most frequent BKV-associated disease after renal transplantation, with BKV reactivation occurring in up to 80% of kidney transplant recipients (KTR). Virological diagnosis of BKVN relies on the detection and quantification of viral load in urine and blood by real-time PCR techniques, allowing preemptive immunosuppressive therapy adaptation. However, the delayed nature and incomplete success of this preemptive strategy underscore the need for prognostic markers of BKV reactivation. Neutralizing antibodies (Nabs) against BKV genotypes were analyzed in a prospective KTR cohort to investigate whether Nabs titers may predict BKV replication. Blood and urine samples were prospectively collected from 168 KTR the day of transplantation, weekly the first month post-transplantation then monthly during 96 weeks. Using the BKV pseudovirus system (Pastrana et al., J Virol 2013), anti-BKV Nabs titers were measured on the day of transplantation and at additional time points post-transplantation. BKV DNA load was quantified in urine and blood samples using a commercial qPCR kit (BK virus R-gene®, Biomérieux, France). BKV strains of KTR displaying viruria and/or viremia were genotyped as previously described (Solis et al, JCM 2016). Anti-BKV Nabs were positive in 164 (97.6%) patients before transplantation. Hundredten (67.1%) KTR harbored higher Nabs titers against genotype I, while 16 (9.8%) and 7 (4.3%) KTR showed higher Nabs titers against genotype II and genotype IV, respectively. Twenty eight KTR harbored higher titers for two genotypes (17 for genotype I and II, 5 for genotype I and IV and 6 for genotype II and IV). Three harbored similar Nabs titers against the 3 genotypes. BKV viruria was detected in 52 (31%) patients 1 to 78 weeks (median 5 weeks) after transplantation. BKV viremia was observed in 28 (16.7%) patients 5–75 weeks (median 18 weeks) after transplantation, among them 13 (7.7%) developed BKVN 10–76 weeks (median 17 weeks) after transplantation. In BKV-replicating KTR, BKV genotype I, genotype II and genotype IV were identified in 45 (86.5%), 1 (1.9%) and 6 (11.5%) patients, respectively. The risk of developing viruria was higher for patients with lower Nabs titers before transplantation against their subsequently-replicating genotype (HR (95% CI) = 0.44 (0.25–0.76; $p = 0.003$). The replicating BKV is acknowledged to be of donor origin. Indeed, donor/recipient mismatches in regard to genotypic neutralization profiles and replicating strains were found to be greater in BKV-replicating KTR ($p < 0.05$). Anti-BKV Nabs titer before transplantation may represent a valuable prognostic marker of BKV replication after

transplantation. Determination of anti-BKV Nabs titer in donors and recipients before transplantation may allow for better-suited induction and maintenance immunosuppressive therapy as well as adapted viral monitoring.

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Abstract no: 341
Presentation at ESCV 2016: Oral 12

A longitudinal study on dynamics of plasma neutralising antibodies and its determinants in HIV-2 infected individuals

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Background: Majority of HIV-2-infected individuals survive as elite controllers. Therefore, HIV-2 infection represents a model for the studies of immune responses that may control an HIV infection, and possibly give leads towards a functional cure. Plasma neutralising antibodies (NAb) are thought to play a central role in HIV-2 evolution and pathogenesis. However, due to relatively silent disease course, it has been almost impossible to diagnose HIV-2 seroconversion time, to follow-up the natural history of infection and to investigate the dynamics of the NAb response. Research group in Sweden and Guinea-Bissau has been organised to investigate the long-term epidemiological trends of HIV-2 infection since 1987. Questions: When does broad and potent NAb response develop in HIV-2 infected individuals? What are the modulators of broad and potent NAb response?

Materials and methods: Forty-six plasma with known T cell count were obtained from 15 individuals from a cohort of police officers in Guinea-Bissau, between 1992-2010. Participants were classified into two subgroups based on mean CD4+ T cell count/μl: Immunocompetent group with cell count ≥500 vs immunosuppressed group with cell count <500 for the neutralization assay ghost 3-ccr5 cell line heat-inactivated plasma and five hiv-2 isolates originating from west Africa were used cut-off point was 30.

Results: Immunocompetent individuals were HIV-2 seroconverted at an earlier age and displayed higher plasma CD8+ T cell count/μl compared to immunodeficient group (median age, 28 years vs 38 years, respectively, $p < 0.05$; median CD8+ T cell count/μl, 755 vs 334, respectively, $p < 0.01$). In all participants, NAb response was found to be potent and broad already during the first year of infection. Moreover, this response persisted throughout the whole follow-up period. Interestingly, at the end of follow-up period, NAb response was significantly broader and more potent in the immunocompetent group compared to immunodeficient group (breadth 4.3 vs 2.9, $p < 0.05$; potency 200000 vs 25000, respectively, $p < 0.05$). In both groups, age at seroconversion correlated negatively with CD4+ and CD8+ T cell count ($r = -0.64$ and -0.41, respectively, $p < 0.05$). In the immunocompromised group, CD4+ and CD8+ T cell counts tended to decrease with infection duration (Spearman's r: -0.62 and -0.88, respectively, $p < 0.05$). Interestingly, decreasing number of cellular immunity cells correlated negatively with potency of NAb response (r: -0.55 and -0.78, $p < 0.05$). In the immunocompetent group, both breadth and potency of NAb response tended to increase with infection duration (r: 0.53 and 0.51, respectively, $p < 0.05$). Furthermore, potency of NAb response correlated positively with CD4+ T cell count (r: 0.72, $p < 0.05$).

Discussion and significance: This study represents the most diverse longitudinal primary infection cohort studied to date for HIV-2 neutralization. Broadly p-NAb response in HIV-1 infection arises only in around 15% of patients after 2-4 years of infection. In addition, p-NAb response tends to fluctuate with low potency. Contrarily, here we show that broad and potent p-NAb response develops in all HIV-2 infected participants during the first year of infection and tends to be persistent. These results may provide insights into the role of potently persistent neutralizing humoral immune response on mild outcome of HIV-2 infection.

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Abstract no: 108
Presentation at ESCV 2016: Oral 13

Implementation of a rapid HIV-1 RNA test in diagnosing acute HIV infections among visitors of the Amsterdam clinic of sexually transmitted infections

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Background: Immediate diagnosis and treatment of acute HIV infection (AHI) is important both from a patient and a public health perspective. Firstly, it can prevent progression to chronic symptomatic HIV disease and thereby improve an individual's prognosis. Secondly, it can reduce the risk of onward transmission associated with AHI in individuals unaware of being infected and usually having high viral loads. At the sexually transmitted infections (STI) outpatient clinic in Amsterdam, the diagnosis of AHI relied on the routine use of serological antibody and antigen assays. These assays have a window phase of at least 15 days between infection and seroconversion. A new promising avenue is the incorporation of a rapid HIV-RNA test that shortens this period with around 5 days.

As part of the HIV-Transmission Elimination AMsterdam (H-TEAM) initiative a rapid AHI diagnosis and referral trajectory was implemented at the STI clinic in Amsterdam in 2015. This involved the addition of a rapid HIV-RNA assay to standard HIV testing among men who have sex with men (MSM). We now present our first experiences with this new rapid AHI test and referral trajectory.

Methods: MSM were assessed for eligibility at the STI clinic for an AHI test. They were either referred by a media campaign (hebikhiv.nl, with a self-referral screening tool), or by their general practitioner (GP), or if they came for routine STI screening. Eligibility was based on symptoms of AHI in combination with condom-less anal sex with a man within 2 weeks to 3 months preceding the visit. Participants completed questionnaires and pro-

vided blood samples (EDTA and serum). A rapid HIV antibody screening assay (Alere Determine™ HIV1/2) was performed. If positive, an established HIV infection was diagnosed. If negative, both an HIV-RNA test (GeneXpert, Cepheid) and a 4th generation HIV antigen/antibody (Ag/Ab) test (Murex HIV Ag/Ab on LiaisonXL) were performed, according to instructions of the manufacturer. If an individual tested HIV-RNA positive but HIV antibody/antigen negative, or indeterminate (defined as only p24 antigen positive), he was considered having AHI. In that case he was offered same day anti-retroviral therapy awaiting confirmation by Western blot.

Results: From August 2015 through April 2016, a total of 142 MSM presented for AHI testing of whom 101 MSM were eligible. Of these 101 men, 59.4% were referred by the website/intervention campaign, 7.9% was referred by their GP and 32.7% via routine screening at the STI clinic. The median age was 32 years (IQR 25–42). All were negative in the rapid HIV antibody test, but 3/101 were indeterminate in the HIV-1 Ag/Ab test, with only p24 antigen positive. All 3were also positive in the HIV-RNA GeneXpert test. These individuals were diagnosed with AHI and referred to an HIV treatment centre the same day, where they were counselled for immediate start of treatment. The other 98 samples were HIV-RNA negative, in concordance with results from the 4th generation Ag/Ab assay. The average turnaround time between intake and test results was 3 h.

Discussion/conclusion: Thus far, 3 individuals were diagnosed with AHI through the referral and rapid AHI test trajectory. Addition of the HIV-RNA rapid test to routine serological testing ensured same day results and immediate start of treatment. The instalment of this trajectory and testing of a larger number of individuals provides the opportunity to evaluate further (cost)effectiveness.

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Abstract no: 129
Presentation at ESCV 2016: Oral 14

Evaluation of HIV-DNA, soluble CD14 and inflammatory markers in HIV-1 positive patients receiving antiretroviral therapy

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Antiretroviral therapy (ART) suppress viral load but, even with long-term effective treatment, HIV infected individuals may have persistent residual viremia (RV) and, low grade inflammation and immune activation that have been associated with non-AIDS defining events. The impact of persistent RV as well as of HIV DNA load on immune activation/inflammation remain unclear.

The purpose of this study was to gain new insights into the relationship between residual viremia, markers of inflammation and the levels of HIV DNA.

Three-hundreds-twenty-one HIV-1 infected patients, from Policlinico Umberto I Sapienza University Hospital, were analyzed retrospectively for 48 months. Patients were grouped according to their viral load (VL) observed during the follow up: Group I: patients with a sustained undetectable viremia ($n = 113$); group II: patients who had at least 2 values of VL detectable but below the threshold value ($n = 113$); group III: patients with at least 2 values of VL over the threshold value but below 200 copies/ml ($n = 95$). Patients had been on ART for a median of 15 years (IQR 9-19 years). HIV RNA load was quantified using the kinetic PCR molecular system (Versant HIV-1 RNA 1.0 kPCR; Siemens Healthcare). Proinflammatory cytokines TNF-α, and IL-6 and microbial translocation marker sCD14 were evaluated by ELISA assay (Enzo Life Sciences). Limit of detection were 15.63 pg/ml, 7.81 pg/ml and 1 μg/ml for TNF-alpha, IL-6 and sCD14, respectively. Quantification of total HIV-1 DNA was performed by using the "Generic HIV DNA Cell" Kit (Biocentric).

There was no difference in the proportion of patients with TNF-α >15.63 pg/ml among groups as well as no difference were detected in TNF levels >15.63 pg/ml. Interestingly, the proportion of patients with IL-6 > 7.81 pg/ml were higher in group I than in group III (35% vs 17%; $p = 0.005$) while IL-6 levels >7.81 pg/ml were significantly higher in group III than in group I [28 pg/ml (IQR 13–45) vs 15.5 pg/ml (IQR 10-30); $p = 0.0047$ by Mann–Whitney with Bonferroni correction; $p = 0.016$ by Kruskal–Wallis]. Significantly lower levels of sCD14 were detected in group I [7.25 μg/ml (IQR 2.7 to <10) and in group II (8.8 μg/ml) (IQR 3.6 to >10)] compared to the median sCD14 level in group III [10 μg/ml (IQR 4 to >10)], as well as significant difference was detected between groups I and II. A higher percentage of patients, with sCD14 levels greater than high limit of quantification (10 μg/ml), was detected in group III compared to group I and II (58% vs 15%, $p < 0.0001$; 58% vs 35%, $p = 0.001$). Again, a significant difference was observed between group I and II (15% vs 35%, $p < 0.001$). The quantification of HIV DNA revealed that in patients with detectable viremia the HIV DNA levels were significantly higher than those detected in individuals with undetectable plasma viremia [group III: 15.3 copies HIV DNA/10^6 PBMC (IQR 12.0–17.1) vs group I: 12.1 copies HIV DNA/10^6 PBMC (IQR 9.4-14.2), $p < 0.0001$; group II: 14.2 copies HIV DNA/10^6 PBMC (IQR 12.4–15.8) vs group I, $p = 0.001$].

In conclusion this study indicated that low/minimal levels of viremia are associated with more elevated levels of sCD14 and IL-6. In addition a higher intracellular viral load wad detected in individuals with low/minimal viremia than in patients showing a full virological suppression. Further studies are needed to carefully establish whether the above markers may represent prognostic indicators of progression of the inflammatory disease.

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Abstract no: 214
Presentation at ESCV 2016: Oral 15

Detection of a novel EV-A71 subgenogroup C1 recombinant variant emerging in Germany, 2015

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Enterovirus A71 (EV-A71) belongs to the family *Picornaviridae* and different genogroups have been described circulating worldwide. It was first detected in 1969 in California and in two outbreaks with severe neurological diseases including polio-like symptoms in Bulgaria (1975) and Hungary (1978). In the Southeast Asia region, EV-A71 is mainly associated with large outbreaks of hand, foot, and mouth disease (HFMD) every year, but in Europe detection of increased EV-A71 circulation associated with meningitis/encephalitis or acute flaccid paralysis has been reported less frequently. Unlike in Asia, HFMD is not a reportable disease in Europe.

The enterovirus surveillance (EVSurv) in Germany is based on investigation of stool and CSF samples from hospitalized patients with suspected meningitis/encephalitis (M/E) or acute flaccid paralysis (AFP) within a laboratory network (LaNED). During enterovirus season 2015, a new recombinant variant of subgenogroup C1 was detected. Overall, 419 stool and CSF samples were tested EV positive (19.5%, 419/2158). Of the 392 strains typed, 43 stools and one CSF sample from M/E patients hospitalized in 25 secondary and tertiary care hospitals from 13 out of 16 federal states in Germany were tested EV-A71 positive (11.2%). Thirty-seven strains were further characterized at the National Reference Centre for Poliomyelitis and Enteroviruses (NRZ PE) by using molecular and virological methods. While 18 strains could clearly be assigned to subgenogroup C2 by the RIVM Enterovirus typing tool (http://www.rivm.nl/mpf/enterovirus/typingtool) based on the VP1 region, 19 strains could not be assigned by the RIVM tool but showed 90–93% nucleotide identity to recently circulating C1 strains in the BLAST search. Amplification and sequencing of the near entire genome and subsequent phylogenetic analyses of the individual genomic regions (5'non coding region, P1, P2, P3) revealed different clustering of the German C1 group in these four dendrograms. No specific amino acid changes in the conserved major antigenic sites were found. However, discrete amino acid changes were identified in the capsid as well as nucleotide changes and deletions within the 5'non coding region.

Our findings underline the need for molecular surveillance of enteroviruses to identify new variants with potential for increased virulence and pathogenicity. During the previous years of EVSurv (2006–2014), C1 strains were only sporadically detected ($n = 8$) and C2 has been the predominant subgenogroup ($n = 168$) of a total of 196/259 EV-A71 characterized. Data from the current enterovirus season will be included in the analyses and it will be interesting to see, whether this new variant dominates or disappears.

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Abstract no: 104
Presentation at ESCV 2016: Oral 16

Preadolescent patients with atypical course of Zika virus (ZIKV) infection: Clinical findings and quantitative viral detection in saliva and plasma

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Zika virus (ZIKV), a mosquito-borne flavivirus, is currently causing a large outbreak in South America, where Brazil is the most affected country. Serious clinical conditions like fetal microcephaly and Guillain-Barre syndrome have already been attributed to ZIKV, yet many aspects of the viral pathogenesis remain unclear. We describe an unusual clinical presentation of ZIKV infection in two preadolescent patients hospitalized in the Emergency Unit of the Clinical Hospital of Ribeirão Preto, University of São Paulo, Brazil. Initially, dengue hemorrhagic fever was suspected in both patients due to the acute myalgia, severe abdominal pain, and elevated hematocrit values. The patients, a male infant with eight years of age and a female 12 years old patient were adynamic and complained of muscular and retro orbital pain, high fever (approximately 39 °C), headache, vomiting and abdominal pain. Additionally, once the male infant complained of a diffuse abdominal pain without specific localization, ultrasound diagnosis of the abdomen was performed, revealing acute mesenteric lymphadenitis. The performed real-time PCR for Dengue and Chikungunya fevers in plasma was negative, and saliva and blood samples were also tested for ZIKV RNA due to the outbreak in the city. Both specimens were positive for ZIKV RNA, as quantitatively the viral load in saliva was higher (median, 2.1×10^3 copies/mL) than in plasma (median, 1.5×10^4 copies/mL). Up to now, the clinical picture described for the ZIKV infection, beyond microcephaly and Guillain-Barre syndromes, includes in general low-grade fever, itching exanthema and conjunctivitis. In these two cases we demonstrate that despite the clinical suspicion of dengue hemorrhagic fever (regional endemicity) the patients were positive for ZIKV. Interestingly, both cases were characterized by vomiting and abdominal pain, in one case accompanied by mesenteric lymphadenitis. These findings point out that ZIKV might be involved in a broader range of clinical symptoms, than previously demonstrated and that differential diagnosis for ZIKV may be performed in pediatric cases demonstrating acute abdominal symptomatology. Moreover, the detection of ZIKV in saliva with a higher viral load than in blood demonstrates that this sample is suitable for diagnosis of the infection in pediatric patients and opens the question for the routes of ZIKV transmission. Financial support: FUNDHERP, FAPESP, CNPq, CAPES.

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[1] German Laboratory Network for Enterovirus Diagnostics.

Abstract no: 289
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Enterovirus D68 diagnosed in severe respiratory and neurological illness in children during 2015–2016 season in Portugal

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Background: Enterovirus D68 (EV-D68) was first isolated in 1962, and since then associated with respiratory illness. The report of severe respiratory and neurological disease including deaths associated to EV-D68 in United States and Canada during August 2014 highlighted the need of epidemiological information regarding EV-D68 circulation. In Europe information was scarce, available only for few countries. In Portugal there was no data available and was critical to know the epidemiology of EV-D68, especially in children hospitalized with severe respiratory or neurological disease. This study aims to identify EV-D68 in Enterovirus positive respiratory samples in children under 18 with clinical diagnosis of severe respiratory infection or neurological illness.

Methods: During 2015/16 winter season, between November/2015 and March/2016, 29 EV positive cases were reported to the National Influenza and Other Respiratory Virus Reference Laboratory (NIC) by two hospitals located in Lisbon and Setubal districts. EV diagnosis was performed in hospitals by biomolecular methods using commercial kits (real time multiplex-PCR, FTD Respiratory pathogens 21 and CLART Pneumovir, Genomica, respectively). EV-D68 was diagnosed by an in house real-time PCR [1]. Virus isolation in RD cell line and phylogenetic analysis of the VP1/VP3 genomic regions will enable the identification of genetic groups in circulation. All samples were irreversibly anonymized. Demographic and clinical data were collected.

Results: EV-D68 was confirmed in 20 respiratory samples previously positive for EV (69%; 20/29). Samples were collected from children with age ranging from 2 months to 6 years old, both genders (9 female; 11 male) with diagnosis of severe respiratory or neurological illness. Eighteen cases were hospitalized (90%; 18/20). Bronchiolitis and pneumonia were the most frequently reported diagnosis, corresponding to 70% (14/20). Two cases have neurologic diagnosis. EV-D68 was identified throughout all study period with the higher number of positive cases detected during January 2016, in week 3. Virus isolation and genetic characterization are under way with expected results in virus phylogeny and evaluation on similarity with recent circulating strains in United States, Canada and European countries.

Conclusions: EV-D68 was detected in a high positive rate (69%) among EV positive cases. This positive rate of EV-D68 was higher compared to the positivity rate of 10.2%, calculated in a European study during 2014 [2]. This finding could be linked to the selection of severe and hospitalized patients in present study, highlighting the involvement of EV-D68 with severe respiratory disease in children. The identification of EV-D68 is also crucial in respiratory samples in children with clinical diagnosis of neurological illness. This study is the first attempt to describe the prevalence of EV-D68 in severe paediatric cases, in Portugal. The strength of EV-D68 surveillance in paediatric and adult population at the national level will be important to understand the epidemiology of EV-D68, age-related susceptibility and association with disease severity.

References

[1] R. Poelman, et al., J. Clin. Virol. 62 (2015) 1–5, http://dx.doi.org/10.1016/j.jcv.2014.11.011.
[2] R. Poelman, et al., J. Clin. Virol. 71 (2015) 1–9, http://dx.doi.org/10.1016/j.jcv.2015.07.296.

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Abstract no: 189
Presentation at ESCV 2016: Oral 18

Evaluation of TTV load kinetics among kidney transplant recipients in the first year post-transplant period

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Introduction: Torque teno virus (TTV) is highly prevalent in humans (90%) with a persistent low-level viremia in the immunocompetent host. Patients who undergo kidney transplant have a high risk of blood-borne viral infections, including the TTV. The objectives of this study are: (i) to assess the level and kinetics of TTV DNA in patients after kidney transplantation; (ii) to investigate the possible association with different conditioning regimens and the kinetics of TTV DNA load; and (iii) to correlate the TTV DNA level with the post-transplant immune reconstitution.

Material and methods: TTVDNA load was assessed in a series of blood samples collected at 15, 30, 60, 90, 180, and 360 days after transplant from 78 kidney transplant recipients (KTRs) prospectively monitored for opportunistic virus infections at the Molecular Virology Unit, Fondazione IRCCS Policlinico San Matteo. The total T-cell, T-CD4$^+$, and T-CD8$^+$ lymphocyte counts were retrospectively retrieved at the same time points used for the TTV DNA load analysis.

Results: In 72/78 (92.3%) patients, TTV DNA was detected at 15 days after transplantation. At 60 days after transplantation, all patients were positive for TTV infection. In 29/78 (37.2%) patients, the peak of viral load was reached at 180 days after transplantation, in 24/78 (30.8%) at 360 days and in 20/78 (25.6%) at 90 days. Only 4 (5.1%) and 1 (1.3%) patients reached the peak of viral load at 60 and 15 days after transplantation, respectively. A significant increase of TTV DNA load was observed between 15 days (median

3.2×10^4 copies/ml) and 180 days after transplantation (median 4.3×10^6 copies/ml; $p < 0.001$). On the contrary, a slight decrease on median TTV load was observed between 180 and 360 days after transplantation ($p = 0.06$). Forty-seven out of 78 (60.3%) patients received basiliximab (Simulect) as induction therapy, while 31/78 (39.7%) received antithymocyte globulin (thymoglobuline). No differences on peak of viral load or time to reach the peak were observed in patients according to different induction therapy. In a series of patients, immunological data were available and therefore compared to TTV load kinetics. After a slight decrease of T-CD4$^+$, T-CD8$^+$ cell counts between 15 and 30 days after transplantation, a significant increase of T-CD4$^+$, T-CD8$^+$ cell counts between 30 and 360 days after transplantation was observed ($p < 0.05$). The kinetics of TTV DNA load and immune reconstitution showed the same tendency but no statistically significant correlation was observed between TTV viremia and lymphocytes levels.

Discussion and conclusions: In conclusion our findings provide evidence of high positivity rate of KTRs during the first post-transplant year. The TTV load kinetics showed a sigmoidal-shaped curve as previously observed by Görzer et al. in lung transplant recipients [1]. No correlation between TTV DNA load level and different induction therapy was observed. Despite the increased level of immune reconstitution, in about 40% of patients the TTV DNA load at 360 days after transplantation was >2 $\log_{10}$ DNA copies/ml with respect to TTV load level in the immediately post-transplant period. Finally, in near 30% of patients the peak of TTV load was observed at 360 days after transplantation.

Reference

[1] I. Görzer, P. Jaksch, M. Kundi, T. Seitz, W. Klepetko, E. Puchhammer-Stöckl, Pre-transplant plasma Torque Teno virus load and increase dynamics after lung transplantation, PLOS ONE 10 (April (3)) (2015) e0122975.

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Abstract no: 344
Presentation at ESCV 2016: Oral 19

Encephalitis caused by a novel adenovirus "orphan genome" in an adult allogenic SCT recipient

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Introduction: Since the addition of a genotype definition to the classical definition of human adenovirus (HAdV) serotypes and the use of next generation sequencing for the differentiation of human adenovirus isolates, the number of HAdV types increased rapidly from 51 to 72. Fifteen of these 21 newly identified types belong to species HAdV-D and 12 of these 15 new HAdV-D types were found to have multiple recombinant genomes. Many of these new HAdV-D types were isolated from the faeces of adult immunocompromised patients suggesting persistent infections of adults and reactivation after immunosuppression. Persistent infections may facilitate co-infections with more than one HAdV-D type and this seems to be a prerequisite for homologous recombination. However, the virulence of most of these new HAdV-D types seems to be rather low (with exception of a few types causing epidemic keratoconjunctivitis, e.g. types 53 and 54) and severe disease manifestations have hardly ever been found to be associated with these types.

Clinical case and diagnostic virology: A 54 year old male presented with encephalitis on day 93 post allogenic stem cell transplantation (SCT). Neurotropic viruses were not detected in CSF but a high concentration of HAdV DNA (3e5 c/ml) was detected by quantitative PCR. In contrast to typical acute infections, HAdV DNA was not detected at other body sites. In peripheral blood, only a low virus load (below the level of quantification, <1e3 c/ml) was found, far too low for a disseminated infection. Therefore, HAdV encephalitis, probably caused by a HAdV reactivation, was diagnosed. In spite of antiviral therapy with cidofovir the patient succumbed to encephalitis on day 122.

HAdV typing: Complete genomic sequencing directly from CSF demonstrated a multiple recombinant HAdV-D genome with a hexon sequence (including the neutralization epitope) almost identical to type 25 whereas other parts of the genome were either related to types 47, 56, 59 or were novel sequence stretches. According to the rules of HAdV taxonomy, the pathogen should be labelled as a new HAdV type. However, virus isolation on A549 cells failed and therefore a new type number was not assigned to this genome.

Discussion and conclusions: Reactivations of species D HAdV may occur in adults as late complications after allogenic SCT. These reactivations can be limited to a single organ (e.g. encephalitis) and thus diagnosis may be difficult. Next generation sequencing facilitates the identification of novel HAdV types, but new complete genomic sequences can also be generated in cases of failed virus isolation resulting in an orphan genome (here defined as a complete genomic sequence without virus isolate). Although the virulence of many HAdV-D types and "orphan genomes" may be low in general, these can cause severe opportunistic infections of immunocompromised patients.

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Abstract no: 250
Presentation at ESCV 2016: Oral 20

Molecular and clinical characterization of Enteroviruses-D68 infections between 2010 and 2015 in Lyon, France using 3D Cell culture and Next-Generation Sequencing

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Introduction: In August 2014, the United States reported an outbreak of Enterovirus-D68 (EV-D68) infections, associated with severe respiratory disease in children. In this study, we investigated the prevalence and molecular evolution of EV-D68 circulating in France between 2010 and 2015, in association with clinical data.

Methods: Respiratory samples collected from patients hospitalized in the University Hospital of Lyon between weeks 31 and 51 from 2010 to 2015 and positive for viruses of *Picornaviridae* family were screened for EV-D68 using quantitative RT-PCR. We further propagated EV-D68 in 3D human primary upper airway epithe-

lial tissues. A RT-PCR was optimized to amplify complete EV-D68 genomes, which were then sequenced using Ion Torrent PGM.

Results: We detected a high prevalence of EV-D68 in 2012 and 2014, with respectively 55 (15.5%) out of 355 and 37 (11.1%) cases detected out of 334 samples tested. In contrast, only six (out of 352) samples were tested positive for EV-D68 in 2010, only one out of 242 samples in 2011, 0 out of 314 samples in 2013 and one out of 417 in 2015. EV-D68 detected in 2012 belonged to both clades A (34.3%) and B (66.7%) while EV-D68 detected in 2014 belonged mainly to clade B (76.5%). We were able to produce most of the EV-D68 complete genomes. Phylogenetic analysis of these sequences reveals the five-year molecular evolution of EV-D68. We also correlate variations observed in the sequences with clinical characteristics of the patients.

Discussion: This analysis may highlight viral polymorphisms associated with mild or severe forms of EV-D68 infection. These polymorphisms could be used as prognosis or diagnosis markers and could be used in clinical management of patient infected with EV-D68.

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Abstract no: 169
Presentation at ESCV 2016: Oral 21

Norovirus molecular epidemiology in a paediatric UK hospital: Unexpected diversity, seasonality and sources of infection

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Norovirus is the leading cause of gastroenteritis worldwide, typically associated with seasonal outbreaks of diarrhoea and vomiting in healthcare institutions and dominated by a single genotype, GII.4.

Using custom-designed norovirus baits for target enrichment (SureSelect) and Illumina sequencing we have sequenced the complete genomes of all norovirus episodes in a paediatric UK hospital over a 19 month period (July 2014–February 2016), consisting of 193 episodes from 186 patients.

We identified a broad range of norovirus genotypes, consisting of 34% GII.P21_GII.3, 22% GII.Pe_GII.4, 16% GII.P4_GII.4, 1% GII.P16_GII.4 and 27% a mixture of GI.P1_GI.1, GI.Pg_GI.1, GI.Pf_GI.3, GI.Pd_GI.3, GI.P3_GI.3, GI.P2_GI.2, GII.P2_GII.2, GII.P7_GII.6, GII.P7_GII.7, GII.P16_GII.17 and GII.P16_GII.17. We did not see the typical winter seasonality expected with norovirus; instead there is a correlation between the number of norovirus episodes and the number of hospital admissions per month ($R = 0.703$, $P = 0.011$). We estimate that movement in and around the hospital accounts for 50% of the variability in the number of norovirus episodes per month. A broad range of genotypes and lack of seasonality is analogous to norovirus epidemiology in the community, rather than the epidemiology seen in non-paediatric healthcare settings.

Maximum Likelihood phylogeny identified 19 sequence clusters suggestive of transmission, involving median 3 patients per cluster (range 2–17). This included clusters that were widely separated in time or location, therefore could not be identified by conventional epidemiological methods. Only 47% of cases were part of a cluster while 53% of norovirus episodes, including some apparently acquired as an inpatient, were sporadic cases with no onward transmission.

We postulate that in this paediatric setting the majority of norovirus episodes are caused by frequent introductions of infection from the community, possibly by infected visitors or staff. In contrast fewer than 50% of cases are associated with nosocomial transmission. These findings have implications for infection control policy in paediatric facilities. Importantly, in addition to established practice of preventing transmission from known in-patient cases, efforts should be focused on identifying norovirus episodes in new admissions and visitors. The diversity of genotypes identified in this study has major implications for vaccine development.

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Abstract no: 66
Presentation at ESCV 2016: Oral 22

Longer duration of viral shedding following infection with a novel norovirus GII.4 strain

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Background: Prolonged viral excretion is common following norovirus gastroenteritis. A key determinant of the duration of shedding is the host immune response. Major new strains of genotype GII.4 virus emerge every two to four years through antigenic drift, and the appearance of antigenically novel strains is associated with more widespread norovirus epidemics. The aim of the present study was to investigate if duration of viral shedding is longer in patients infected with such emerging, epidemic GII.4 strains, as compared to patients infected with previously circulating strains

Materials and methods: We conducted a prospective cohort study during three consecutive norovirus seasons (2010–2013), which included patients hospitalised with community-onset norovirus gastroenteritis. Patients with concurrent bacterial infection or immunosuppression were excluded. Norovirus was diagnosed with real-time PCR and subtype strain was determined from capsid sequence (ORF2), via phylogenetic tree analysis. Follow-up samples were obtained weekly until four weeks after inclusion. Rapid clearance was defined as a negative sample for norovirus by day 14, and slow clearance was defined as shedding of norovirus RNA for more than fourteen days. Proportions were compared with Fisher's exact test.

Results: Novel epidemic strains appeared twice during the study period; GII.4-2010 (New Orleans) in the first season, and GII.4-2012 (Sydney) in the 2012-2013 season. We included 24 patients from these two seasons, in whom duration of shedding could be determined and a GII.4 virus was found. Median age was 83 years (range 25–99) and 50% were women.

The majority of patients ($n = 19$) were infected with viruses that clustered with the respective season's epidemic strain, whereas five patients were infected with previously circulating subtypes (GII.4-2008 or older in 2010/11; GII.4-2010 or older in 2012/13). Slow clearance was observed in 1/5 (20%) patients infected with previously circulating strains. This proportion was significantly higher among patients infected with an epidemic strain, where 16/19 (84%, $p = 0.01$) remained PCR-positive for more than two weeks.

Conclusion: In this limited pilot study, we found that a long duration of viral shedding appears to be more common in patients infected with novel, epidemic norovirus GII.4 strains. This may be

due to lack of pre-existing immunity. Conversely, non-protective immunity from previous exposure to circulating GII.4 subtype strains may reduce the duration of shedding.

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Abstract no: 73
Presentation at ESCV 2016: Oral 23

Molecular characterization of long-term shedding of respiratory syncytial viruses isolated from consecutive samples collected in haematological patients

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Objective: Respiratory syncytial virus (RSV) is a frequent cause of upper respiratory tract infections, but can be associated with severe and prolonged infection in immunocompromised patients. The objective of this study was to investigate the genetic variability of RSV in haematological patients with prolonged RSV infection.

Methods: To inform for infection control measures during the winter seasons, haematological patients at the University Hospital Heidelberg are routinely screened for respiratory viruses on admission to a ward or presentation in the outpatient department. In RSV positive patients, screening was continued on a regular basis until three consecutive samples were tested negative. Nasopharyngeal swabs (NPS) were screened for influenza, parainfluenza and RSV using rtPCR during the winter seasons 2011-2013. In patients with prolonged RSV infection (defined as viral shedding ≥28 days), Sanger sequencing of the second hypervariable region of the RSV G gene was performed in consecutive samples. Further, ultra-deep sequencing (UDS) by next-generation-sequencing was performed in representative samples (three each with the shortest and longest viral shedding) to identify further RSV variants. Readily available medical records were retrospectively reviewed to obtain basic characteristics, clinical and laboratory data.

Results: In total 16 patients with prolonged RSV A infection were analysed (median shedding 79.5 days, 81.2% male). Phylogenetic analysis identified RSV strains as genotype NA1 (2011/12) or ON1 (2012/13). Almost all patients showed identical sequences of the G gene at the beginning compared to the end of the shedding period ($n = 14/16$). However, in two patients with particularly long viral shedding (333 and 142 days) Sanger sequencing revealed the presence of mutations leading to premature stop codons. The G protein was thereby shortened by between 60 and 71 amino acids. In addition, UDS revealed RSV strain variants (67%) with premature stop codons at different positions in a patient with the second longest viral shedding period (155 days). All three patients with premature stop codons received intravenous immunoglobulins. Interestingly, UDS revealed also a loss of the 72nt duplication with no further changes in strain variants of the ON1 genotype in 5 of 6 patients.

Conclusion: Long-term shedding of RSV in haematological patients showed only minor changes of RSV strains and is thereby not likely caused by re-infections. However, long shedding periods and lack of immune selective pressure in the immunocompromised host seems to allow the virus to strip a part of the C-terminus of the G glycoprotein. To the best of our knowledge, the loss of the characteristic 72nt duplication in ON1 variant strains is here described for the first time.

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Abstract no: 92
Presentation at ESCV 2016: Oral 24

Plasma Torquetenovirus (TTV) DNA load as a surrogate marker of reconstitution of CMV-specific immunity in the allogeneic stem cell transplantation setting

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Background: Torquetenoviruses (TTV) are small circular ssDNA viruses classified within the family *Anelloviridae*. TTV DNA can be detected in plasma of most individuals at variable levels. Transplant recipients are particularly prone to carry high TTV burdens in blood. The dynamics of TTV viremia in allogeneic stem cell transplant recipients (Allo-SCT) were previously shown to correlate with that of graft reconstitution, suggesting that TTV replicates mainly in hematopoietic cells. In this context, we investigated whether monitoring of TTV DNAemia early after transplant permits to infer the level of CMV-specific T-cell reconstitution following Allo-SCT.

Material and methods: The patients included ($n = 23$) in the study underwent Allo-SCT at the University Clinic Hospital of Valencia between 2013 and 2014. CMV DNAemia was monitored weekly by a RealTime PCR (Abbott Molecular, Des Plaines, IL). The quantification of TTV DNA was performed by a real-time Taqman assay, using serial dilutions of standards TTV (from 10^2 to 10^7). The PCR targeted a segment of the untranslated region of the viral genome: forward primer 5′-GTGCCGIAGGTGAGTTTA-3′, reverse primer 5′-AGCCCGGCCAGTCC-3′ and probe 5′-TCAAGGGGCAATTCGGGCT-3′. The detection of TTV vas carried out in plasma samples obtained at baseline and on days 21, 30 and 60 after transplant. Enumeration of CMV pp65 and IE-1-specific gamma interferon-producing CD8+ and CD4+ T cells was performed on day 30 after transplantation by flow cytometry for intracellular cytokine staining.

Results: TTV DNA was detected in 22 out of 23 patients (95.7%). At baseline (median day −7, range −8 to +7) TTV DNA load values ranged between 1.59 $\log_{10}$ to 7.97 $\log_{10}$ (median 3.08 $\log_{10}$). Baseline median TTV DNA levels were comparable regardless of the conditioning regimen used. Overall, median TTV DNA levels were not related to the immunossupressive regimen used for graft vs. host disease prophylaxis. A total of 14 (60.9%) patients developed an episode of CMV infection during the first 100 days after transplantation (median 31 days, range 5–61). Plasma TTV DNA levels at baseline and on day 21 were comparable in patients with or without subsequent CMV DNAemia ($P = 0.74$). In general there was an increase in the TTV load over time, which paralleled that of total

lymphocyte counts. A significant correlation was found between the TTV DNA load and the number of CD4+ and CD8+ T cells on day 30 (σ Spearman 0.68 and 0.71, respectively, $P = 0.001$). Notably, there was a strong correlation between plasma TTV DNA load and CMV pp65 and IE-1-specific IFN-γ-producing CD4+ and CD8+ T cells on day 30 (σ Spearman 0.80 and 0.58, respectively, $P = 0.003$). In fact, ROC analyses showed that a cutoff of 3.90 $\log_{10}$ of TTV viremia predicted the reconstitution of CMV-specific CD8+ T cells at levels >1 cell/μL (previously shown to be protective from the occurrence of CMV DNAemia) with a PPV of 75% and a NPV of 86.6% ($P = 0.006$).

Conclusion: Plasma TTV DNA load may serve as a surrogate marker for reconstitution of the CMV-specific CD8+ T-cell response. Larger studies are nevertheless needed to draw definitive conclusions on this issue.

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Abstract no: 229
Presentation at ESCV 2016: Oral 25

Whole-genome sequencing of adenovirus in immunocompromised paediatric patients directly from clinical samples elucidates molecular epidemiology of suspected outbreaks

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Background: Adenoviruses are significant pathogens for the immune supressed, with 15% of paediatric bone marrow transplant (BMT) recipients experiencing adenovirus reactivations. Adenoviraemia post-BMT is associated with increased treatment costs, longer hospital stays, and increased morbidity and mortality. Whole-genome sequencing of adenovirus provides a wealth of clinically relevant information on genome variation, including phylogeography, resolution of locally circulating subtypes and improved molecular epidemiology, the capacity to identify new recombinant subtypes, and mutation data in response to antiviral drug selection pressure. Adenovirus-positive clinical specimens from patients at Great Ormond Street Hospital for Children include isolated and suspected outbreak cases.

Methods: We retrospectively identified 90 adenovirus positive residual diagnostic samples (including blood, urine, swabs (eye, throat and unspecified), ascetic fluid and endo-tracheal aspirate) with virus loads greater than 5×10^4 copies/ml from 30 patients treated at Great Ormond Street Hospital (GOSH) collected between 1st January 2015 and 30th April 2016. Additional samples from a suspected outbreak of adenovirus at GOSH in 2011/2012 and three cultured reference strains (D9, E4 and F40) were also sequenced and analysed. A set of custom RNA baits designed against 350 full and partial human adenovirus genomes to capture the full diversity of the Mastadenoviruses was used to enrich adenoviral DNA during library preparation (SureSelect target enrichment). DNA extracts from clinical samples were sequenced on the Illumina MiSeq platform without prior culture or specific PCR amplification. Genome mapping or *de novo* assembly were performed using CLC Genomics Workbench 8.

Results: Genome coverage ranged between 20 and 100% (median coverage 89%). Nearly-whole genomes were recovered from 56 out of 96 clinical samples, at mean depths ranging from 7 to >7000×, including one sample with an estimated genome input of 1200 copies. *De novo* assembly of cultured isolates produced consensus sequences identical to the reference sequences for these strains. We were able to genotype all clinical isolates on the basis of similarity to reference adenovirus genomes, including samples for which PCR-based typing had previously failed. No mixed infections (multiple adenovirus genotypes within the same sample) were identified in this sample cohort. Whole genome sequences were used to elucidate molecular epidemiology within GOSH.

Conclusions: Using target enrichment we have successfully performed whole-genome sequencing of adenovirus directly from clinical samples, avoiding the mutagenic aspects of PCR amplicon-based sequencing or the loss of diversity associated with culture. We have genotyped clinical adenovirus samples in a single reaction. Further sequencing of naso-pharyngeal aspirate and faecal adenovirus samples is ongoing. We are using whole-genome sequences to inform molecular epidemiology and infection control at GOSH. This work significantly increases the number of adenovirus whole genome sequences from clinical isolates currently available, which may aid in future vaccine designs.

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Abstract no: 290
Presentation at ESCV 2016: Oral 26

Seroprevalence of hepatitis E virus among the Portuguese general population and prevalence of silent infection in blood donors – HEPeCONTROL

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Introduction: The discovery of autochthonous hepatitis E in industrialized countries has changed the understanding of hepatitis E virus (HEV) infection in these regions, now known to be mainly due to zoonotic transmission of HEV genotype 3 via consumption of undercooked contaminated pork meat. Furthermore, the HEV seroprevalence that has been described in European countries, as well as the high rate of asymptomatic HEV infections has led to the concern about transfusion-transmitted HEV infections. Studies carried out on European blood donors have reported HEV acute infections, with viraemia (HEV RNA) ranging from 0.08% to 0.14%. In Portugal, no nationwide survey on HEV seroprevalence has been done, and the risk of blood donations from HEV silent infected donors has never been reported.

Aims: The present study aimed to provide detailed information on the seroprevalence of HEV infection in the Portuguese population and evaluate silent HEV infection in Portuguese blood donors. This study is part of HEPeCONTROL project (60DT2) under EEA grants funding.

Materials and Methods: Sera from a representative cohort of the Portuguese population ($n = 1656$) distributed by geographic location (30 territorial units), and age (0–99 years of age) collected between July 2015 and February 2016 were tested for the presence of anti-HEV IgG by EIA (*recom*Well HEV IgG, version 2015, Mikrogen). Plasma from blood donors ($n = 2115$) of SSMT CHUC collected

between October to December 2015 was tested for both anti-HEV IgM and HEV RNA. They were evaluated by EIA (*recom*Well HEV IgM, version 2015, Mikrogen) and in case of anti-HEV IgM positive or equivocal results the samples were retested by immunodot assay (*recom*Line HEV IgM, version 2015, Mikrogen). Nucleic acid was extracted from each plasma sample (400 μl) using Nucleic Acid Isolation Kit I (MagNA Pure Compact system, Roche Diagnostics) and HEV RNA was detected using an in house real-time RT-PCR (Jothikumar and colleagues, 2006) and two commercial real-time RT-PCR kits (*ampli*Cube HEV 2.0, Mikrogen and RealStar HEV 1.0, Altona).

Results: An overall HEV IgG seroprevalence in Portuguese population of 16% was found with seropositivity increasing significantly with age ($p < 0.05$). Differences between regions were also observed ranging from 8% to 28%. From the total of 2115 blood donors, 7 (0.33%) were found positive for anti-HEV IgM. HEV RNA was detected in 19 (0.90%) blood donors by the three real-time RT-PCR used. These plasma samples were negative for both anti-HEV IgM and IgG. HEV RNA concentration of plasma samples ranged from 2.0×10^4 to 8.4×10^1 IU/ml when calculated by a WHO/IS strain standard curve ($r^2 = 0.998$).

Conclusions: This study provides insight in the exposure of the Portuguese general population to HEV and generates information on risk profiles regarding demographic data (age and region of residence). The prevalence of HEV silent infection in Portuguese blood donors was 1.2% based on the presence of IgM and HEV RNA. This high prevalence could be the due to the high volume of plasma used for acid nucleic extraction and the screening of unpooled samples.

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Abstract no: 322
Presentation at ESCV 2016: Oral 27

Assessment of the Illumina MiSeq massively parallel sequencing platform for simultaneous analysis of Hepatitis C virus resistance to all direct-acting antivirals combination regimes

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Background: Interferon-free direct-acting antivirals (DAA) combinations regimens are highly effective for the treatment of chronic hepatitis C virus (HCV) infection. However, DAA regimes are often specific to a particular HCV genotype, and the re-treatment of HCV patients with failure to DAAs remains challenging, as a result of the emergence of resistance associated variants (RAVs). Resistance testing prior to treatment was not previously recommended. However, new consensus guidelines are now incorporating resistance testing for the management of DAA failures. From the available next-generation sequencing (NGS) platforms, the Roche-454 (FLX or Junior) are being discontinued in 2016, and there is a need for new HCV resistance NGS assays.

Methods: We evaluated the Illumina MiSeq platform for the simultaneous sequencing of the three HCV genes target of the currently approved and phase III DAA combinations: NS3(protease), NS5A and NS5B. In a pilot, validation study, the three HCV genes from 45 HCV-genotype 1b infected patients (DAA naïve) were amplified by RT-PCR. The different amplicons corresponding to the same patient were pooled equimolarly before library preparation. Patient-specific indexed paired-end libraries were obtained with the Nextera XT DNA Sample Preparation Kit and the Nextera Index Kit. After normalization, all libraries were pooled and sequenced in parallel using the MiSeq Reagent Kit v2 (300 cycle) in a MiSeq system. To determine the error rates of the process, a plasmid with a cloned HCV-NS3 protease was also processed in the run.

Results: We obtained 136 Megabases, with 94.5% of reads passing the error filtering algorithms (Nasu et al. PLoS ONE, 2011), and 92% mapping to the regions of interest, with similar coverage (mean; range = 7660; 759–18,729). The overall error rate for the procedure was 0.319%, which is similar to that obtained previously with the same HCV-NS3 protease clone in the 454 platform (Salvatierra et al. Virologie, 2013). Using a majority rule consensus sequence for each patient sample, several natural RAVs were found at different prevalence. For NS3: A87V (2.3%), R117C/H (2.3% each), S122N/T (2.3% each), V170I (25%) and S174A (2.3%). For NS5A: R30Q (9.1%), L31I (3.3%), Y93H (4.5%). For NS5B: L159F (22.7%), C316N (38.7%), C316H (2,3%), S368A (2.3%), Y448H (4.5%) and S556G (15.91%). No RAVs were found in NS3-Q80, R155, A156, D168, M175; NS5A-L28, P58; or NS5B-S282, S365, N411, M414, G554 or D559.

Conclusions: The Illumina MiSeq platform allows for the simultaneous sequencing of HCV NS3, NS5A and NS5B regions, with a high coverage and multiplexing capacity. Althoug clinically-relevant variants may be represent frequencies >15% of the quasispecies, after filtering the error rates obtained with the MiSeq are similar to the Roche-454 platform, thus potentially allowing for detecting minority variants down to 1% of the viral population. While major resistance mutations to protease and nucleosidic inhibitors are rare in our isolates, we detected the NS5A-R30Q and Y93H major RAVs, and the NS5B-L159F accessory and C316N RAVs. Because the concept HCV treatment is being updated to finding the right combination of DAAs in a particular patient, obtaining the complete DAA susceptibility profile with this platform will be useful to further treatment optimization and for the management of DAA failures.

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Abstract no: 107
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Hepatitis D virus infection in Slovenian patients with chronic hepatitis B virus infection: A national prevalence study

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Background and objectives: Of 350 million individuals chronically infected with hepatitis B virus (HBV) worldwide, approximately 15 million have been exposed to hepatitis D virus (HDV) infection. As a result of vaccination against HBV, the prevalence of HDV has decreased in the last 20 years in the majority of European countries, especially in Southern Europe. However, it recently begun to rise again in some European countries, like France, Germany and the United Kingdom, due to immigration from endemic areas (mainly from Africa, Eastern Europe and Turkey). Slovenia is a country with a population of approximately two million and an estimated HBV prevalence of less than 5%. No reports on HDV prevalence in Slovenia have been published to date in the peer reviewed literature. The aim of our study was therefore to determine the HDV prevalence in Slovenian patients with chronic HBV infection.

Materials and methods: Our study included 1305 HBsAg-positive serum samples from the same number of patients randomly selected from HBsAg-positive patients referred to the Slovenian national reference laboratory for viral hepatitis between February 1998 and December 2015. Considering the 95% confidence interval and 2.5% margin of error, our sample size was representative for all patients with chronic HBV infection in Slovenia. Serum samples were retrospectively tested for the presence of total anti-HDV antibodies using commercially available ETI-AB-DELTAK-2 (DiaSorin, Saluggia, Italy). Anti-HDV-positive samples were further tested for the presence of anti-HDV IgM antibodies and hepatitis D antigen (HDV-Ag) using commercially available ETI-DELTA-IGMK-2 (Diasorin) and ETI-DELTAK-2 (Diasorin), respectively. Additionally, the in-house HDV reverse-transcription real-time PCR, enabling amplification of a 71-bp fragment of the conserved genomic region encoding HDV-Ag (*J Clin Microbiol 2005;43:2363-9*; *J Clin Microbiol 2010;48:2022-9*) and with the analytical sensitivity of at least 300 viral copies/ml, was used to determine the presence of HDV RNA in anti-HDV-positive samples.

Results: Total anti-HDV antibodies were detected in three out of 1,305 tested samples (0.23%; 95% confidence interval 0.08-0.67%). Several consecutive serum samples were collected from all three anti-HDV-positive patients. Apart from total anti-HDV antibodies, no other HDV infection markers were detected in any of the tested samples collected from a 48-year old male patient, indicating that the patient has recovered from the past HDV infection. On the contrary, anti-HDV IgM antibodies and HDV RNA were detected in all tested samples obtained from a 28-year old female patient and a 60-year old male patient, suggesting the ongoing chronic HDV infection.

Conclusions: In the first national prevalence study the observed prevalence of HDV infection among HBsAg-positive patients in Slovenia was surprisingly low, considering the fact that Italy, with the HDV prevalence of 8.1%, is one of Slovenian neighboring countries. Due to the observed low prevalence of HDV infection, routine testing for HDV should not be considered in differential diagnosis of exacerbation of liver disease in Slovenian patients with chronic HBV infection.

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Abstract no: 167
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Antiviral effect of interferons on BK virus infection

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The human polyomavirus BK (BKV) is a ubiquitous pathogen that establishes an asymptomatic persistent infection in the urinary tract of 80% of the human population. In immunocompromised patients, reactivation of the BKV infection is the cause of nephropathy and hemorrhagic cystitis. Diseases associated with BKV infections are increasing at the same time as potent immunosuppressive therapies are developing. This highlights the importance of components of the immune system in controlling viral reactivation. However, the immune response to BKV, particularly the role of antiviral cytokines in infection control is poorly documented.

Here, we investigated the antiviral effect of interferons (IFN) on the BKV infection in renal cells. We tested IFN-alpha, lambda

and gamma on the Dunlop strain of BKV in Vero cells and 293FT cells. Treatment with IFN-gamma inhibited the expression of the viral late protein VP1 in a dose-dependent manner and decreased the expression of the early and late viral transcripts. A weaker antiviral effect was observed with IFN-alpha and IFN-lambda. These results are associated with a prolonged STAT1 phosphorylation with IFN-gamma but not with IFN-alpha and lambda. The difference of efficacy between these two types of interferon suggests that some interferon induced proteins, only produced by IFN-gamma had an antiviral effect on BKV infection. Transcriptome analysis reveals that six proteins could be involved in this specific antiviral effect and are under investigation.

In conclusion, the most potent IFN on BKV infection is the IFN-gamma. Finding the action mechanism of this effect could help to develop a therapeutic strategy.

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Abstract no: 231
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High incidence of ganciclovir-resistant cytomegalovirus infections in solid organ transplant patients

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Introduction: Human cytomegalovirus (CMV) is a pathogen in immunocompromized individual such as recipients of solid organ transplants. These patients are monitored for CMV-DNAemia, i.e. the presence of DNA in plasma, and treated when CMV becomes detectable or when symptoms of CMV disease occur. CMV-seronegative recipients of organs from CMV-seropositive donors have a very high risk of developing CMV infections. CMV-seropositive recipients may develop reactivations, or possibly reinfections.

First line treatment of CMV infections is intravenous gancicolvir or oral valgancicolvir. Studies have shown that antiviral resistance may develop in up to 10% of treated individuals (Chou et al., 1999). Resistance testing should be performed when there is doubt about treatment response. This is usually done by sequencing the targets for ganciclovir, the protein kinase and the viral polymerase gene, UL54. In this study we determined how frequently CMV resistance occurred in our hospital, which is the largest organ transplantation center in the Netherlands.

Methods: Stored plasma samples of patients with CMV DNAemia were retrospectively investigated for antiviral resistance by sequencing the UL97 and UL54 genes. All patients who had DNAemia at levels >10 000 copies/ml plasma for more than 2 weeks were included. Sequencing was performed according to methods described before (Scott et al., 2004).

Results: 35 patients undergoing treatment for CMV disease were included with a median of 3 samples per patient (range 2–9). In 13 (37%) patients with mutations were detected known to be associated with resistance. In 12 (34%) patients no mutations were detected. In 10 (29%) patients mutations were detected which were not previously described. Resistance associated mutations occurred more frequently in patients with high levels of CMV DNA (>100 000 copies/ml) ($P = 0.005$). High levels of CMV-DNA were more frequently observed during primary CMV infections.

Conclusions: Our research shows that in patients with CMV DNA levels of >10 000 copies, the incidence of antiviral resistance is nearly 40%. Further research with a larger number of patients is needed to investigate factors associated with a higher risk of developing antiviral resistance.

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Abstract no: 116
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A multidrug resistant HSV1 infection occurring under cidofovir treatment for ADV infection in an immunocompromised child: Perspectives to new antiviral drugs

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Resistance of herpes simplex viruses (HSV) to conventional antiviral drugs acyclovir (ACV) and foscarnet (FOS) are an increasing concern in immunocompromised patients and particularly bone marrow transplant patients. Research in antiviral drugs leads to the emergence of new therapeutic classes, as inhibitors of the helicase-primase complex (HPI), pritelivir or amenamevir (Burrel et al., 2014; Tyring et al., 2012). These new molecules represent potential optional treatment for herpes infections even for combination therapy due to synergistic effect when used with ACV (reviewed in James and Prichard, 2014).

We report a 16-year-old immunocompromised adolescent diagnosed with an acute myeloid leukemia (LAM2, FAB classification). He had an infection with a systemic adenovirus (ADV) and presented a period of 5 months of aplasia after chemotherapy and ADV infection. He underwent allogeneic hematopoietic stem cell transplantation (HSCT). The HSV1 infection was firstly located at mucocutaneous site with a voluminous lower lip lesion, then followed by a systemic infection with a viral load up to 5100 copies/ml. During the same period, the patient developped an ADV and BK virus co infection. He received intravenous ACV and cidofovir (CDV), associated with intravesical CDV. Sequence analysis of the full-length *UL23* encoding thymidine kinase gene, *UL30* encoding DNA polymerase gene and *UL5* encoding helicase gene were performed. No mutation was found on *UL23* and *UL5*. Unfortunately, S724N located on *UL30* gene, known to confer multi resistance to ACV, FOS and CDV was detected. In regards to this multidrug-resistant strain, an authorisation for amenamevir use was also requested and accepted by the French ANSM. The clinical evolution of the HSV disease was favourable, as the lower lip lesion regressed and the blood PCR HSV1 became negative. Nevertheless, this young immunocompromised patient finally died from a multiple organ failure.

Current treatment strategies lead to expose regularly immunocompromised patients to antiviral treatments. The selection of multidrug-resistant strains could be explained by the circumstances of antiviral overexposure, used even for other viral infection. New antiviral drugs are deeply needed in order to manage

such cases, and HPI appear to be an option for treatment of resistant HSV strains to conventional antivirals (Himaki et al., 2012).

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Abstract no: 117
Presentation at ESCV 2016: Oral 32

HIV neutralising antibody delivered by gene therapy with a stable retroviral vector encoded in baculovirus expression systems

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Introduction: Virus like particles (VLPs) are replication-incompetent virus shells that represent an intact, non-replicative virion lacking a genome. They maintain the original antigenic composition of the packaging component incorporated into the virion's outer membrane. Recently, interest in using VLPs as gene therapy agents has increased [1].

In this study, we are aiming to develop retrovirus like particles to serve as a new gene therapy carrier system. Our VLP is of simian immunodeficiency virus (SIV) origin and to extend the limited cell tropism inherent in SIV the VLP will be pseudotyped with vesicular stomatis virus (VSV) glycoprotein. The particle will deliver IgG1 b12 antibody genes for insertion into the mammalian genome, to produce long-lasting, high titres of neutralising anti-HIV monoclonal antibody [2,3].

Baculoviruses can be used as vehicles to efficiently deliver and express genes in mammalian cells. BacMam technology uses a recombinant baculovirus engineered to contain a mammalian expression cassette for transgene expression in mammalian cells. The mammalian gene is expressed without baculovirus replication. VLPs can be produced using this expression system [2,4].

Methods: Five different target genes have been cloned into five altered transfer plasmids, to construct five different recombinant baculoviruses containing the Tat/Gag/Pol genes of SIV, plus the VSV glycoprotein gene and T7 polymerase. Either CMV or T7-promoters are driving expression of all genes. Confirmation of cloning was done by restriction digests using unique restriction enzymes followed by sequencing. Recombinant baculoviruses are generated by homologous recombination between virus baculovirus DNA and the transfer plasmids. Mammalian cells will be transduced with recombinant baculoviruses to express proteins of interest. Western blot and Elisa confirm detection of protein expression.

Results: Thus far, all genes of interest have been cloned successfully into a baculovirus transfer vector (pOET6 BacMAM).

Discussion: We believe that the BacMam construct will deliver SIV genes into mammalian cells and produce SIV like particles psuedotyped with VSV glycoproteins. Psuedotyping the SIV like particles with VSV-G can eliminate any limitation caused by the use of SIV envelope genes, by widening cell tropism. Thus IgG1 b12 antibody genes will be delivered and inserted into the genome of numerous cell types, to produce long-lasting, high titres of neutralising anti-HIV monoclonal antibody. Since baculoviruses cannot replicate in mammalian cells, this system can be used in vivo as well as in vitro.

Reference

[1] C.M. Thompson, et al., Critical assessment of influenza VLP production in Sf9 and HEK293 expression systems, BMC Biotechnol. 15 (2015) 31.
[2] T.A. Kost, J.P. Condreay, D.L. Jarvis, Baculovirus as versatile vectors for protein expression in insect and mammalian cells, Nat. Biotechnol. 23 (2005) 567–575.
[3] T. Nakajima, K. Nakamaru, E. Ido, Development of novel simian immunodeficiency virus vectors carrying a dual gene expression system, Hum. Gene 1874 (2000) 1863–1874.
[4] S. Shukla, C. Schwartz, K. Kapoor, Use of baculovirus BacMam vectors for expression of ABC drug transporters in mammalian cells, Drug Metab. 304–312 (2012).

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Abstract no: 152
Presentation at ESCV 2016: Oral 33

European non-polio enterovirus surveillance and laboratory detection – Are we prepared to detect an enterovirus outbreak?

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Background: Enteroviruses (EVs) are known to cause large and severe outbreaks, as recently demonstrated by EV-D68 in USA and Europe. Another type, EV-A71, is also known for its ability to cause geographically widespread and clinically significant hand, foot, and mouth disease (HFMD) outbreaks. Although EV-A71 outbreaks have been mostly described in Asia so far, the virus is already known to circulate in Europe and has been occasionally linked to the fatal outcomes. We have evaluated the European preparedness for detection and characterisation of non-polio EVs in order to improve our response for (re)-emergencing EVs linked to severe disease.

Methods: An on-line survey on non-polio enterovirus surveillance and enterovirus typing/characaterisation was submitted to all EU/EEA Member States (MS) national coordinating competent bodies.

Results: A total of 29 MS from 30responded to the survey. Twenty-seven countries conduct non-polio enterovirus surveillance based on reporting of enteroviruses detected from clinical specimens, two of them without further characterisation of EV-positive samples. Almost all countries include typing of EV-positive samples obtained from individuals with neurological infections ($n = 24$) in their EV surveillance, whereas HFMD and respiratory infections are included in the EV surveillance less frequently ($n = 18$ and 16, respectively). Three countries have also initiated specific surveillance for HFMD, and eleven for EV-D68. EV-D68 surveillance has been established via sentinel influenza surveillance ($n = 7$), by typing EV-positive respiratory samples ($n = 10$) and/or via acute flaccid paralysis (AFP) surveillance ($n = 5$). Virus isolation is performed in all except one country, whereas molecular methods are used by all. Non-polio enterovirus typing is performed in 26 MS; ten MS type/characterise only culture-positive EV isolates, whereas the remaining MS subject also PCR-positive samples to typing/characterisation. Nineteen MS have introduced sequencing based EV typing, whereas neutralisation assay is used by 16 MS and seven of them rely entirely on it. Over 5000 EV-positive specimens were successfully characterised/typed in the EU/EEA region in 2015. The estimated number of typed EV specimens was <50 in eleven countries (including all seven countries that type by neutralisation assay only), whereas six MS had successfully typed over 300

EV specimens in 2015 mostly by sequencing. Survey revealed issues related to EV typing/characterisation mostly in those countries which rely only on virus neutralisation.

Discussion: Our survey demonstrated some form of non-polio EV surveillance throughout the EU/EEA region but only partial HFMD and EV-D68 surveillance activity in the region. Virus isolation is known to be less sensitive for EV detection than molecular methods, and depends on cell lines used. It should also be noted that most emerging EV types are refractory to isolation and antibodies used in neutralisation assays are unlikely available. Hence it is important to aim for introduction of molecular methods for EV typing/characterisation throughout the region. We will develop European algorithms for non-polio EV identification to strengthen the European enterovirus laboratory preparedness.

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Abstract no: 43
Presentation at ESCV 2016: Oral 34

Neurotrophic potential of Saffold virus and the effects of its leader protein in the central nervous system of a mouse model

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Background: Saffold virus (SAFV) is a human cardiovirus belonging to the *Picornaviridae* family. Since its discovery in 2007, 11 genotypes of SAFV have been identified and it has been shown to be ubiquitous and causes infection early in life. Information about SAFV infection tends to be concentrated on respiratory and gastrointestinal tract infections, however, there has been increasing interest in SAFV infection of the central nervous system (CNS) due to clinical cases of SAFV with neurological symptoms (Nielsen et al., 2012; Zhang et al., 2015) and also its close relation to Theiler's murine encephalitis virus (TMEV). Compared to TMEV, SAFV has a truncated Leader (L) protein, a protein essential for the establishment of persistent CNS infections.

Methods: In order to study the replication kinetics and pathogenesis of SAFV and its L protein, we generated an infectious cDNA clone of SAFV and chimeric SAFV containing L gene of TMEV DA strain. For *in vivo* pathogenicity study, we established a mouse model for SAFV infection and then studied SAFV and chimeric SAFV infections in the central nervous system.

Results: We showed that both SAFV and chimeric SAFV are able to infect Vero and Neuro2a cells well, but only chimeric SAFV was able to infect RAW264.7. We then showed that AG129 mice lacking IFN-α/β and IFN-γ receptors provide a good animal model for SAFV infection, and further identified the locality of the infection to the ventral horn of the spine and several locations in the brain. Next, we showed that SAFV infects both neuronal and glial cells in the brain, causing apoptosis in both. Lastly, we showed that SAFV does not cause persistent infection nor demyelination in this model.

Conclusion: Overall, our results provide a strong basis on which the mechanisms underlying Saffold virus-induced neuropathogenesis can be further studied.

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Abstract no: 175
Presentation at ESCV 2016: Oral 35

Torque Teno Virus in liver tranplant patients in Scotland, UK

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Torque Teno Virus is a small (approximately 3.8 kb) single stranded DNA virus first identified in a patient with non A-G hepatitis. Despite the circumstances of its discovery, its role as an aetiological agent of hepatitis remains uncertain and it has been shown to be a widely prevalent virus globally, including amongst health individuals. However, previous studies have found a higher viral load in immunosuppressed patients, such as those receiving post-transplant immunosuppression, and it has been suggested that TTV viral load could prove a useful indicator of immunosuppression. Given this and TTV's anecdotal association with hepatitis, a study was undertaken to assess the TTV viral load of liver transplant patients in comparison to control patients. Serum samples from patients attending the Scottish Liver Transplant Unit ($n = 93$, $M{:}F$ 1:0.89, age range; 22–81 mean age = 57) and samples from non-hepatic patients attending Glasgow Royal Infirmary ($n = 50$, $M{:}F$ 1:3.54, age range; 19–86 mean age = 61) were assayed for TTV using a pan-TTV qPCR and genotyped using Genogroup specific conventional PCRs. Whilst it was found that transplant patients have a higher mean viral load in comparison to the control group (3.8×10^6 vs 1.0×10^4) the difference was not statistically significant ($p = 0.9007$). In addition, variables such as age, sex, time since transplant and reason for transplant were assayed for significance but no correlation with viral load was identified. This lack of significance may be due to the sample size and work is ongoing to expand this. The genogrouping assays showed that all 5 Genogroups were present in both the liver transplant patient and control groups. However, Genogroup 3 was the most common in the liver transplant patient group whilst Genogroup 4 was the frequently identified Genogroup in the control group. The significance of this is being further investigated, with a correlation between Genogroup and viral load being one possible explanation. The differences recorded in viral load between the liver transplant patient and control group, and the various variables within the patient group, deserve further investigation, in particular as previous studies have reported statistically significant increased viral loads in patients receiving other forms of solid organ transplant and it would be beneficial to identify if liver transplant patients show a similar pattern or not. Also further investigation into the various patient subgroups could elucidate the route of transmission of TTV and its pathogenic role, if any. Lastly the variation in Genogroup incidence between the patient and control group is an interesting and novel finding that warrants further research.

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Abstract no: 96
Presentation at ESCV 2016: Oral 36

Differences among mumps virus surface proteins between genotype G and other genotypes and their potential effect on mumps virus immunity and pathogenesis

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Background: Mumps used to be a mild childhood disease, but recent outbreaks mainly affected vaccinated young adults. One hypothesis on the recurrence of mumps suggests that there might be a mismatch between the surface proteins (fusion protein (F) and hemagglutinin-neuraminidase (HN)) of the Jeryl Lynn vaccine strain and the circulating genotype G strains. These surface proteins are important for mumps virus immunity and pathogenesis. In this study, we therefore compared the variable positions in the F and HN proteins between the Jeryl Lynn vaccine strain and wild type mumps virus strains, including genotype G.

Methods: Sequence information of the Jeryl Lynn vaccine strain, genotype G strains and other wild type strains were collected from UniProt and from clinical samples collected at the RIVM. HN and F sequencing of the clinical samples was performed at the RIVM with six primers as described previously [1]. As no protein structures of the F and HN protein were available for the mumps virus, we have built homology models of the HN and F protein with the aid of FoldX. The templates used were 4JF7 and 4GIP, respectively. We have subsequently used the in-house developed Scop3D tool to visualize the sequence conservation/variation on both protein structures [2].

Results: For the F and HN protein, we found that variations occur in functional or immunological important regions (known B-cell epitopes, glycosylation sites, etc.). Differences between the Jeryl Lynn vaccine strains and genotype G were found in known B-cell epitopes for both the F and HN protein and in glycosylation sites of the HN protein. Additionally, the variations in genotype G in these regions, seem to be very specific, as these variations change only to a limited number of other amino acids. Of special interest is the variations found in amino acid positions 400–402 and 464–466 of the HN protein, as these positions are theoretical glycosylation sites.

Conclusion: In this study, we have identified multiple variable sites in the mumps virus surface proteins which could affect mumps virus immunity and pathogenesis. Difference in functional or immunological important regions of the proteins, such as known B-cell epitopes and glycosylation sites, could result in a mismatch between the Jeryl Lynn vaccine strain and circulating genotype G strains, as antibodies raised through vaccination with the Jeryl Lynn strain might not be able to neutralize the circulating genotype G strains. This could possibly contribute to the recurrence of mumps outbreaks in vaccinated young adults.

Reference

[1] S. Gouma, J. Sane, D. Gijselaar, J. Cremer, S. Hahné, M. Koopmans, et al., Two major mumps genotype G variants dominated recent mumps outbreaks in the Netherlands (2009–2012), J. Gen. Virol. 95 (Pt 5) (2014) 1074–1082.

[2] T. Vermeire, S. Vermaere, B. Schepens, X. Saelens, S. Van Gucht, L. Martens, E. Vandermarliere, Scop3D: three-dimensional visualization of sequence conservation, Proteomics 15 (8) (2015) 1448–1452.

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Abstract no: 350
Presentation at ESCV 2016: Oral 37

Structural basis of Zika virus cross-reactivity and cross-neutralization with flavivirus post-infection and post-vaccination sera

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The explosive spread of Zika virus to Pacific Islands as well as South- and Middle America underscores the potential threat of newly emerging arthropod-borne (ARBO) viruses. The evidence of transplacental infections and associated malformations of newborns (microcephalies) led the WHO to declare a Public Health Emergency of International Concern on February 1, 2016. At present it is unclear which factors contributed to this emergence, which may include mutational adaptations that changed the character of this virus. In addition, however, there is evidence that pre-existing flavivirus antibodies may play a role in Zika pathogenesis. This is especially important in the context of other flaviviruses that co-circulate in Zika-endemic regions.

We have therefore conducted a systematic analysis of Zika virus cross-reactivity and cross-neutralization of serum samples from people after infections with heterologous flaviviruses, including dengue, West Nile and tick-borne encephalitis (TBE) as well as after yellow fever, Japanese encephalitis and TBE vaccinations. The data are presented in the context of amino acid sequence divergence between flaviviruses and the recently determined atomic structure of the Zika virus envelope protein E. The implications of these findings for the specific serodiagnosis of different flavivirus infections will be discussed.

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Abstract no: 87
Presentation at ESCV 2016: Oral 38

Zika virus infections in travellers and contacts in Lombardy, Northern Italy 2016

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Objectives: Zika virus infections in patients returning to Lombardy Region (Northern Italy) and contacts were investigated.

Methods: serum samples of patients with potential Zika virus (ZIKV) infections were tested for the presence of specific IgM and IgG antibodies (Anti-Zika virus ELISA (IgM) and Anti-Zika virus ELISA (IgG) by Euroimmun, Lübeck, Germany). Furthermore, the presence of specific ZIKV antibodies was confirmed by plaque reduction neutralization test (PRNT). Serum, saliva, urine and semen samples, collected during the acute phase, were examined for the presence of ZIKV RNA with two methods: a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) targeting a conserved region of ZIKV and a pan-Flavivirus heminested RT-PCR targeting a conserved region of the NS5 gene followed by sequencing of amplicons.

Results: in the period 18 February–20 April 2016, 5 confirmed cases of Zika virus infection were diagnosed in Lombardy Region (10 million inhabitants) in Northern Italy. Four (1 female and 3 male) patients had an history of recent travel, 1 arrived from the Dominican Republic, 1 from El Salvador and 2 from Brazil, while 1 patient, the wife of the patient returning from the Dominican Republic, had not travelled. Thus, a sexual transmission was documented. The patient returning from El Salvador was a 41 years old pregnancy woman, 8 weeks gestation. During the symptomatic phase of the infection were collected and analyzed for each patients serum/plasma, saliva, urine and semen in males. Zika virus-RNA was detected in 5/5 (100%) urine samples of the five patients, in 4/5 (80%) saliva samples while the viral genome was identified in 2/5 (40%) plasma samples. Furthermore, in 3/3 (100%) semen samples was detected Zika virus-RNA. In the symptomatic period Zika virus specific IgM were detected in 3/5 (60%) patients while 0/5 (0%) specific IgG were detected. Zika virus-RNA has been detected in serum up to 54 days, in urine up to 40 days, in saliva up to 11 days and in semen up to 19 days after onset of symptoms.

Conclusions: Zika virus infection shows a prolonged persistence in peripheral blood with the potential of autochthonous spread to competent mosquitoes. In addition, the presence of virus in semen is an additional factor for autochthonous infections. Thus, sexual partners of travelers must be included in surveillance protocols.

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Abstract no: 81
Presentation at ESCV 2016: Oral 39

Zika virus infections imported to Portugal, the National Reference Laboratory experience: The importance of sample collection time lapse in diagnosis

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Zika virus (ZIKV) belongs to the genus *Flavivirus* and was first isolated from the blood of a febrile sentinel rhesus monkey during a study of yellow fever in 1947, in Zika Forest, Uganda. ZIKV is transmitted by *Aedes* sp. Mosquitoes, as Dengue, Yellow fever and Chikungunya viruses, and was until recently considered a mild pathogenic mosquito-borne flavivirus with very few reported human cases of self-limiting acute febrile illnesses most often with maculopapular rash, headache, malaise and conjunctivitis, all followed by full recovery without sequel. During 60 years, cases of ZIKV infections were detected only sporadically in Africa, and South and Southeast Asia.

In 2007, the epidemiological history of ZIKV started to change with a substantial outbreak in Yap Island (Federated States of Micronesia), followed, since 2013, by outbreaks in French Polynesia and other Pacific Islands. In French Polynesia a link relating ZIKV infections with the increased incidence of Guillan-Barré syndrome and other neurological complications was assumed mainly in regions with previous dengue epidemics. Nonetheless, at this time, no one was prepared for the dramatic outcomes of ZIKV arrival in Brazil and, the explosive spread in the country and the huge increase of several congenital malformations, microcephaly, and other neurological disorders, including Guillain-Barré syndrome and acute disseminated encephalomyelitis (ADEM) described since 2015. In February 1, 2016, the World Health Organization (WHO) declared ZIKV infection a Public Health Emergency of International Concern.

Genetic studies enable the identification of three distinct genotypes: West African (Nigerian cluster), East African (MR766 prototype cluster) and Asian. All recent reported ZIKV outbreaks have been associated to the Asian genotype. As most pathogenic flavivirus, only a small percentage of ZIKV cases (estimated in *ca.* 25%) are symptomatic, and transmission via transfusion of infected blood or organs donations, or sexual transmission, remains a risk. The presence of other flaviviruses endemic in the same geographic range is also a fact to take in consideration, as the incoming proves that the eventual existence of antibodies to another flavivirus might facilitate a worsen development in ZIKV infection. On the other hand, the clinical similarity of Zika and dengue virus infections and the cross-reactivity of Zika antibodies with dengue viruses (DENV) might have enabled the incorrect association of several Zika infections to DENV and certainly difficult serologic diagnosis.

Here, we discuss the clinical and laboratory aspects related to some of the imported human cases of Zika virus in Portugal mainly from Brazil, discuss the importance of time lapse in the choice of sample and diagnostic analysis to achieve a confirmed ZIKV diagnosis result. Particularities of the diagnosis of secondary infections by ZIKV after a probable primary DENV infection and the probable ZIKV sexual transmitted infection in Madeira Island will be presented.

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Abstract no: 103
Presentation at ESCV 2016: Poster 1

Direct fluorescent antibody, focus diagnostics Simplexa™ Flu A/B & RSV and multi-parameter customized respiratory Taqman® Array Card testing in immunocompromised patients

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Background: Molecular assays for diagnosis of influenza A, influenza B, and respiratory syncytial virus (RSV) with short turnaround time are of considerable clinical importance. These viruses are clinically most important since they are the most frequently encountered, since they cause substantial disease burden and since a targeted treatment and prevention exists. In addition, rapid and accurate diagnosis of a large panel of viral and atypical pathogens can be crucial for an appropriate and sometimes life-saving clinical management in immunocompromised patients.

Objectives: The goals of the present study are firstly to compare the sensitivity and specificity of direct fluorescent antibody (DFA) (Argene and Light Diagnostics), Simplexa™ Direct assay system and a customized Taqman® Array Card (TAC) testing for RSV, Flu A, and Flu B. Secondly to compare the cost, turnaround time (TAT) and diagnostic yield of different algorithms for the detection of respiratory pathogens in this group of immunocompromised patients. And finally to test the Simplexa™ assay on BAL samples, which has only been validated on nasopharyngeal swabs (NTS) so far.

Study design: We collected 125 NTS and 25 BAL samples from symptomatic immunocompromised patients. Samples for which Simplexa™ and TAC (premarket version Cambridge-Brugge) results were discordant underwent further verification testing using the multiplex real-time PCR assay FTD Flu/HRSV (Fast-track Diagnostics), on the nucleic acid extract that was used for TAC testing. The TAC assay is based on singleplex, reverse transcription real-time PCR, targeting 24 viruses, 8 bacteria and 2 fungi simultaneously.

Results: As expected, the overall sensitivity was significantly lower for DFA testing than for the two molecular methods ($p < 0.05$). However, when considering results for each pathogen separately, the difference in performance between DFA and molecular methods was only statistically significant for Flu A. The Simplexa™ direct test missed one RSV, one Flu A and two Flu B positive samples in comparison to the TAC assay and verification PCR. One sample was found strongly positive for Flu A by Simplexa™ (Ct 15), but was negative by viral culture, TAC and verification testing. Nevertheless, the differences in individual and overall sensitivity and specificity of Simplexa™ testing were not significant compared to TAC testing ($p > 0.1$). For BAL samples only ($n = 25$), the sensitivity and specificity of the Simplexa™ assay was 100%.

In total, DFA identified 14 samples (9.3%) and Simplexa™ testing found 24 (16%) samples positive with one pathogen each. The TAC assay identified 93 samples with one or more respiratory pathogens (62%). More than half (54%) of Simplexa™ negative samples were positive by TAC for other pathogens than RSV, Flu A and B. A co-infection rate of 15.3% was found by TAC.

The estimated costs and TAT were 8.2€ and 2 hours for DFA, 31.8€ and 1.5 hours for Simplexa™ and 56€ and 6 h for TAC testing.

Conclusions: Based on these results, performing a first line molecular method such as the Simplexa™ test instead of DFA would be necessary to obtain an acceptable overall sensitivity, albeit at a higher cost generated in the laboratory. Performing the TAC as a second line test for patients with a negative Simplexa™ result would increase the diagnostic yield significantly, albeit at an even higher cost.

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Abstract no: 106
Presentation at ESCV 2016: Poster 2

Correlation between a new BioPlex® 2200 ToRC IgM assay and established commercial assays for the detection of IgM antibodies to *T. gondii*, rubella, and cytomegalovirus

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Background: Toxoplasmosis, cytomegalovirus (CMV) infection, and rubella are diseases that can cause serious complications during pregnancy. Traditionally, laboratories test for antibodies to these diseases using manual methods such as enzyme immunoassay (EIA). EIA can be associated with challenges such as low specificity, irreproducibility, low throughput, and high demand for labor. As laboratories are moving towards a centralized and automated core lab, there has been a transition away from manual testing towards fully automated test systems.

The improved Bio-Rad BioPlex 2200 ToRC IgM assay is a multiplex flow immunoassay, currently in development, employing an array of *T. gondii*, Rubella, and CMV antigen coated beads as individual substrates in order to simultaneously detect antibodies reactive to these pathogens in a single reaction vessel using a single sample. Since the BioPlex 2200 substrates are segregated by unique fluorescent signatures, the presence of specific antibodies can be individually determined in a single test.

Methods: Samples from a presumptive positive population (Toxo IgM $n = 152$, Rub IgM $n = 133$, and CMV IgM $n = 153$), a test ordered sample population (Toxo IgM $n = 259$, Rub IgM $n = 322$, and CMV IgM $n = 325$), a pregnancy sample population (Toxo IgM $n = 479$, Rub IgM $n = 477$, and CMV IgM = 476), and samples from a normal population (Toxo IgM $n = 941$, Rub IgM $n = 940$, and CMV IgM $n = 941$) were analyzed with the new BioPlex 2200 ToRC IgM assay. The *T. gondii* IgM results were compared to the BioMerieux Vidas Toxo IgM assay. The rubella and CMV IgM results were compared to the original BioPlex 2200 ToRC IgM assay. The improved BioPlex 2200 ToRC IgM was further evaluated for imprecision around the cutoff.

Results: Among the presumptive positive sample population, the BioPlex 2200 ToRC IgM assay showed a positive agreement of 96.6%, 100.0%, and 100.0% for Toxo IgM, Rub IgM, and CMV IgM respectively. The negative agreement for the combined pregnancy and test ordered sample population was 98.2%, 99.9%, and 99.6% for Toxo IgM, Rub IgM and CMV IgM respectively. The negative agreement for normal population samples was 99.6% for all three analytes. The prevalence of IgM antibodies in the normal population samples was 0.6%, 1.6%, and 2.0% for Toxo IgM, Rub IgM, and CMV IgM respectively. The imprecision for positive samples was shown to be between 5.0% and 9.4% for Toxo IgM, 4.8% and 11.6% for Rub IgM, and 5.0% and 11.8% for CMV IgM. The standard devi-

ation of results from the negative samples was <0.1 SD across all three analytes.

Conclusions: The data demonstrate that the BioPlex 2200 ToRC IgM results are comparable to other commercially available assays. Furthermore, the simultaneous detection and identification of antibodies to *T. gondii*, Rubella, and CMV allows laboratories to increase throughput and improve overall workflow.

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Abstract no: 109
Presentation at ESCV 2016: Poster 3

Comparison of the Beckman Coulter DxN VERIS and Abbott RealTime assays in analyzing HCV positive plasma samples

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Background: The accurate determination of viral load in Hepatitis C infected patients is very important for treatment monitoring and efficacy. The DxN VERIS Molecular Diagnostics System has become commercially available more recently and the VERIS HCV assay is performed on this fully-automated, random-access system. This study was conducted to assess the precision, linearity and sensitivity of the VERIS HCV assay. A direct comparison between the VERIS HCV assay and the Abbott RealTi*m*e HCV assay was also performed which included frozen plasma samples from individuals on HCV treatment.

Methods: Four HCV quality controls, diluted to nominal concentrations of 1.56, 3.38, 5.0 and 7.9 log IU/mL, and one negative control were tested on DxN VERIS in duplicate for 20 days. For linearity analysis, one high viremic sample (>10,000,000 IU/mL) was diluted to several concentrations to demonstrate the linearity of the VERIS HCV assay. Analytical sensitivity was also determined for the VERIS HCV assay using 4th WHO traceable material and the result calculated using Probit analysis (95% hit rate). For method comparison 100 HCV-positive clinical specimens with viral loads ranging from 12 to 22 million IU/mL were tested on both systems. Additionally, 80 frozen EDTA-plasma specimens derived from 20 individuals undergoing HCV treatment on both systems and viral load profiles generated.

Results: Coefficients of variation calculated from the DxN VERIS results ranged from 1.55% for the highest concentration to 9.4% for the lowest nominal concentration in precision analysis. All HCV negative samples were confirmed to be undetectable. The linearity was validated for range of 1.7–6.7 log IU/mL and showed a good correlation ($R^2 = 0.988$). With an analytical sensitivity of 6.2 IU/mL (CI95%: 5.1–7.9 IU/mL) VERIS showed similar sensitivity to Abbott. In Bland-Altman analysis both assays showed an overall mean difference of 0.245 log IU/mL (VERIS HCV – RealTi*m*e HCV, $n = 91$). The correlation co-efficient based on 91 qualified results (9 results were excluded from analysis as they were outside the linear range of either assay) was 0.982 (slope 1.11, intercept −0.19).

The results of 80 frozen EDTA-plasma specimens, covering 4 different blood collection time points, showed a good overall agreement in the viral load profiles obtained for genotype 1–3. For genotype 4 ($n = 4$) the mean difference between both assays results were −0.96 log IU/ml (Veris minus RealTi*m*e) in this analysis.

Conclusions: The VERIS HCV assay demonstrated a good correlation over the linear range, particularly at the lower end of the linear range. The limit of detection was comparable to that reported for the Abbott RealTi*m*e assay. Sensitivity and precision of both assays were comparable on a high level in general analysis. Discrepancies between both assays were measureable higher at viral load above 3 log IU/ml. Results received from the VERIS assay tended to be higher quantified than results received from the RealTi*m*e assay. However, genotype 4 isolates in clinical samples were lower quantified as compared to RealTi*m*e. With random access and time to first result of about 105 min the VERIS system is faster and less time-consuming than the Abbott RealTi*m*e System.

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Abstract no: 118
Presentation at ESCV 2016: Poster 4

Characterisation and standardisation of material in QCMD EQA programmes in the absence of higher order standards

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Background: Diagnosis and subsequent monitoring of infectious disease levels after treatment using quantitative molecular assays is well established in clinical practical for a range of different viral pathogens including HIV, HCV, HBV, CMV, EBV, and BK. Within EQA programmes for these pathogens, quality assessment requires assessing laboratory performance in both the detection and quantitation of the target pathogens relative to their respective peer group and where an International Standard is available in the same common units. For these type of programmes EQA has always calibrated the material it uses against the International Standard. However for the material of infectious diseases there are currently no international standard or certified reference material hence QCMD develops and uses internal reference materials to characterise the materials used within the EQA regardless of whether the EQA programme is 'qualitative' or 'quantitative'. By doing this we ensure consistency within and across EQA programmes over time.

In this study the use of digital PCR (dPCR) to quantify materials for two EQA programmes that are currently primarily qualitative (herpes simplex virus 1 (HSV1) and Varicella Zoster virus (VZV)) was examined and compared to reference quantitative real-time PCR (qPCR) methods.

Methods: Pathogen specific materials were prepared for use within each respective EQA programme. Each target pathogen HSV1 and VZV was prepared transport media. The samples were characterised using both qPCR based methods and digital PCR (Bio-Rad QX200 droplet dPCR) by selected reference testing laboratories. The samples were then included in the EQA panels and distributed to laboratories in two challenges across 2015 to registered participating laboratories. Laboratories were asked to treat the materials as they would a clinical sample. Results were returned to QCMD via a dedicated online system where sample results including quantitative data along with information on the assay workflow were collected.

Results: Data analysis was performed on all quantitative datasets HSV1 (106), VZV (86). Datasets were categorised based on the amplification assay manufacturer and method. Outliers were assessed through the application of Grubbs' analysis to each assessment group. Datasets were considered suitable assessment if more than five laboratories reported data using the same assay manufacturer after removal of outliers. This allowed a consensus concentration to be derived. Each assessment groups datasets were

compared against the overall consensus the reference qPCR method and the dPCR assigned values.

The overall commercial consensus for the HSV1 sample was 3.8 Log_{10} copies/ml with qPCR 3.7 Log_{10} copies/ml and a dPCR result 4.1 Log_{10} copies/ml. The overall commercial consensus for the VZV sample was 3.7 Log_{10} copies/ml with qPCR 3.3 Log_{10} copies/ml and a dPCR result 3.2 Log_{10} copies/ml.

Conclusions: A comparison of the results show that dPCR results aligned with the quantitative values determined using commercial assays and the in-house qPCR assay despite an international standard not being available. Digital PCR is advantageous allowing quantitation without the need for a calibrated standard the data presented here indicates that this is a useful method to calibrate EQA material allowing standardisation of the samples and allowing comparison of results between laboratories.

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Abstract no: 120
Presentation at ESCV 2016: Poster 5

Performance of the Aptima® HBV Quant assay on the fully automated Panther® system

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Background: The Aptima HBV Quant Assay is a quantitative assay on the fully automated Panther system that recently received CE-ICD approval. The assay uses real-time Transcription-Mediated Amplification (TMA) technology and targets two regions of the HBV genome to achieve high sensitivity and accurate quantitation. This CE-IVD assay is intended for monitoring HBV DNA in plasma and serum specimens as an aid in the management of patients with chronic HBV infections undergoing antiviral therapy. Here we describe validation studies of the assay.

Methods: The 95% Limit of Detection (LoD) of the assay was determined by Probit analysis using dilutions of the 3rd HBV WHO International Standard (NIBSC 10/264) and HBV positive specimens (genotype A to H) in HBV negative human plasma and serum. Lower Limit of Quantitation (LLOQ) was established for each genotype by diluting clinical specimens and the 3rd HBV WHO International Standard in HBV negative human plasma and serum. Specificity was determined using 292 fresh and 747 frozen specimens from normal blood donors (521 plasma specimens and 518 serum specimens). The linear range of the assay was established by dilution of HBV DNA in plasma and serum at concentrations ranging from 0.86 to 9.26 log IU/mL. Linearity for genotype A to H was established by dilution of linearized HBV DNA in buffer at concentrations ranging from 1.44 to 8.44 log IU/mL. Precision was determined using a 28 member panel made by diluting HBV positive clinical specimens or spiking HBV DNA into negative plasma and serum. A method comparison was conducted against the Abbott RealTime HBV assay using 614 clinical specimens from HBV infected patients.

Results: The 95% LoD using the 3rd WHO standard was 5.6 IU/mL for plasma and 4.3 IU/mL for serum. The 95% LOD across 8 genotypes was determined to be 6.4 IU/mL or lower for plasma and 7.3 IU/mL or lower for serum. The LLOQ for the assay was 10 IU/mL. Specificity was 99.9% with 95% confidence intervals of 99.5–100% for serum and plasma data combined. The assay demonstrated a linear range of 1–9 log IU/mL with good linearity across the range of quantitation for all genotypes. Precision was less than 0.23 log SD across the range of the assay for both serum and plasma. Deming regression of quantitative results in 614 clinical specimens for Aptima HBV Quant compared to Abbott RealTime HBV resulted in a slope of 1.05, an intercept of −0.14, and an R^2 of 0.99.

Conclusions: The Aptima HBV Quant assay on the fully automated Panther system is a highly sensitive and specific assay with a broad dynamic range for quantitative detection of all HBV genotypes. The assay results are highly correlated to those from Abbott RealTime HBV assay. The performance of the Aptima HBV Quant assay makes it an excellent candidate for sensitive monitoring of HBV DNA in plasma and serum specimens.

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Abstract no: 122
Presentation at ESCV 2016: Poster 6

Verification of the Argene® real time PCR kits on the new eMAG™ extraction platform

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NucliSENS® easyMAG® (bioMérieux) is one of the extraction platforms currently claimed in the instructions of use of ARGENE® real time PCR assays (bioMérieux). It's successor bioMerieux' new extraction platform, named eMAG™ (*), provides full automation of sample extraction starting from primary tubes while keeping well established NucliSENS® easyMAG® chemistry. Automation of the extraction (primary sample tube management, automated addition of internal control, automated addition of silica, eluate transfer) requires adaptation of the current NucliSENS® easyMAG® extraction protocols to "eMAG™ extraction methods".

Suitability of eMAG™ as nucleic acid extraction platform for ARGENE® real time PCR assays will be verified in performance studies demonstrating equivalency between easyMAG® and eMAG™. Flexibility of the laboratory workflow will be ensured by harmonization of extraction methods available to the user on eMAG™ in order to allow processing of several sample types in the same extraction run.

The first part of the results from verification studies performed in order to claim eMAG™ as suitable extraction platform for different ARGENE® real time PCR assays will be presented. In particular, a focus will be made on the Legio pneumo/Cc r-gene® assay (ARGENE® range, bioMérieux), which will be the first CE marked kit to claim the eMAG nucleic acid extraction.

(*) Not yet commercialized.

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Abstract no: 123
Presentation at ESCV 2016: Poster 7

External assay controls – How do you monitor yours?

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For both serological and molecular diagnostic laboratories work is performed within a quality system, typically ISO 15189. As a result, laboratories are required to use and monitor an external run control. These controls provide an independent control which is not

restrained within a particular batch, and gives a more challenging sensitivity than that seen with internal controls. Monitored over time and across different assay batches, these controls provide reassurance that the assay is as sensitive as it can be, which ultimately leads to accurate and consistent patient diagnosis and treatment.

Inter and intra laboratory variation can lead to erroneous results. It is recognised that a laboratory may achieve relatively consistent results from run to run but there can still be differences between operators, local equipment, pipetting technique and even calibration and training. Different methods exist to gather intra laboratory data from complex excel sheets to hand written lab note books. However it is harder and requires laboratory collaboration to gather inter-lab data. This too should be monitored overtime and if a deviation is seen it is acted upon and investigated to maintain sensitivity and accuracy. Similarly comparing one laboratories data against another is insightful. With this information a laboratory can assess why their data may differ from the consensus and investigate local procedures to rectify such anomalies.

Many laboratories rely on EQA schemes to enable this comparison, whilst an excellent way of comparing performance across a large range of laboratories and assays, such schemes only provide a periodic snap shot of performance.

The National Institute for Biological Standards and Control (NIBSC) has developed a web-based Result Reporting System (RRS), for the data monitoring of its serology and NAT quality control (QC) reagents. Through the provision of Intra-lab charts and Inter box plots. It allows real time intra and inter-laboratory comparison and monitoring and by applying Westgard Rules to the data any deviations from the norm are flagged, thus this software can provide an early warning sign that a laboratories assay or equipment is failing.

Aided by a demonstration of RRS, this presentation will outline the need for the use of external control material over solely using internal controls and will importantly highlight the necessary monitoring needed in order to ensure reproducibility and consistency of assay results.

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Abstract no: 124
Presentation at ESCV 2016: Poster 8

CMV Run control r-gene® (ARGENE® range, bioMérieux): A tool to ensure the reliability of human cytomegalovirus nucleic acid amplification technique results

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The primary aim of any laboratory is the timely delivery of reliable results with a minimum of errors, maintaining confidence in the results for all stakeholders. The use of independent quality control (IQC) provides constant and consistent monitoring of an assay results in a systematic manner so that variation in the assay system can be monitored over time (day-to-day test variation, lot-to-lot performance of test kits, operator variation, etc.). An IQC material must be robust, stable and well characterized. Its properties should be as close as practically possible to patient specimens and should be processed throughout the analysis in the same way as the clinical sample.bioMérieux has developed an IQC named CMV Run Control r-gene® (ARGENE® range)*. Its routine use enables monitoring run to run performance for human cytomegalovirus nucleic acid amplification technique (NAT) assays for human clinical samples. CMV Run Control r-gene® is intended for health care professional and for *in vitro* use only.

This CMV Run Control r-gene® (ARGENE® range) consists in a non-inactivated whole CMV strain (AD169) spiked in pooled human plasma tested negative for CMV, HIV, HCV, HBV, Parvovirus B19, and EBV. This formulation allows to mimic naturally occurring specimens containing CMV DNA. The CMV Run Control r-gene® (ARGENE® range) has no assigned concentration value but it is defined in order to be within the dynamic range of most molecular assays. This control should therefore be validated for use as a run control and the expected results determined by the end user for their particular CMV NAT assay, extraction and instrument combination.

Performance results obtained in-house (precision and stability studies) of CMV Run control r-gene® (ARGENE® range) established with the CMV R-gene® kit (ARGENE® range, bioMérieux) on the platform combination NucliSENS® easyMAG® (bioMérieux)/ABI 7500 Fast (Life Technologies™) will be presented.

* Not yet commercialized.

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Abstract no: 126
Presentation at ESCV 2016: Poster 9

RNA internal control, a new tool for the rapid development of detection tools by real time PCR in outbreak situation. Application to the detection of Middle East Respiratory Syndrome human coronavirus

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Since the emergence of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012 in the Arabian Peninsula, many questions remain unanswered on modes of transmission and reservoirs of virus. The MERS-CoV causes severe respiratory illness. The epidemic origins are uncertain but probably linked to a zoonosis. The bat or the camel are discussed as reservoirs of the virus. Globally, since September 2012, WHO has been notified of 1,714 laboratory-confirmed cases of infection with MERS-CoV, including at least 618 related deaths (WHO report – 14 April 2016).

In such outbreak situations, especially with emerging organisms causing severe human diseases, it is important to quickly develop a test for the detection the virus involved.bioMérieux developed a generic kit, RNA internal control r-gene® (bioMerieux), combining an internal control and a core kit to be used in combination with either proprietary or commercial primers and probes. This tool associated with specific primers and probes constitute a ready-to-use duplex premix for the detection of targeted RNA in a sample.

On this principle, bioMérieux developed a real-time PCR assay for the rapid detection of MERS-CoV. A set of primers and probe and a transcript used as positive control (MERS-HCoV primers r-gene® – RUO #20-010 and MERS-HCoV probe r-gene® – RUO #20-011, MERS-HCoV transcript – RUO #68-010, bioMérieux) were designed on the S gene, coding for the spike structural protein.

The internal control, added before the extraction step, allows to check simultaneously extraction efficiency and presence of inhibitors. Extractions were performed on NucliSENS® easyMAG®

(bioMérieux) followed by amplification on 7500 Fast Real-Time PCR System Dx (Applied Biosystems®).

Results of analytical sensitivity, exclusivity and inclusivity studies are presented below.

Analytical sensitivity was determined on the whole system using *in vitro* transcript spiked in respiratory samples. This study showed a 95% limit of detection at 2.89 $\log_{10}$ cp/mL of sample [IC 95%: 2.65–3.29] i.e. 780 cp/mL of sample [IC 95%: 450–1950].

Exclusivity was confirmed with the major human respiratory viruses including other human coronaviruses. No cross-reaction was observed. The QCMD Panel MERS-CoV 2015 was tested and results are as expected, Core and educational samples were detected. Among the 19 other commercial kits, all but one gave also the correct identification. The bioMerieux's solution targeting S gene gave equivalent results than the kits targeting upE or N gene.

The combination of RNA internal control r-gene® ready-to-use premix with the MERS-CoV primers r-gene®, MERS-CoV probe r-gene® and MERS-CoV transcript r-gene® (research use Only) is a good candidate solution for the detection of MERS-CoV virus and demonstrate the interest and reliability of the RNA internal control for the rapid development of detection tool, in outbreak situation.

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Abstract no: 131
Presentation at ESCV 2016: Poster 10

Multicentre evaluation of the variability of adenovirus quantification by PCR

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Background: Viral load measurements using nucleic acid amplification techniques (NAT) are critical for the diagnosis and management of human adenovirus (HAdV) infections. A variety of laboratory-developed tests (LDT) and commercial assays are used. The aim of this study was to evaluate variability in the quantification of HAdV by NAT, and the effectiveness of candidate HAdV reference materials to harmonise viral load measurements.

Methods: HAdV positive patient samples, including; whole blood, plasma, serum, urine, stool, eye swab, nasal lavage and sputum, were sourced from clinical laboratories and typed by sequencing. Virus stocks representing the 9 HAdV types identified in the clinical samples (Types 1, 2, 4, 5, 7, 14, 31, 40, 41), were grown in Hep2C cells. The HAdV DNA concentration in the clinical and cultured virus samples was determined at NIBSC using a commercial and LDT.

Study samples comprised cultured virus representing 9 HAdV types in 10 mM Tris–HCl (pH7.4) containing 0.5% human serum albumin (TCS1-9), clinical samples diluted in HAdV-negative sample matrix, and a dilution series of cultured virus prepared in HAdV-negative sample matrix (TCS-matrix samples, these represented the same HAdV type and matrix as the clinical samples). Twelve laboratories from 6 European countries took part in the study. Each laboratory tested TCS1-9, and the clinical and TCS-matrix samples relevant to their quantitative HAdV NAT assay.

Results: In total, 16 datasets were received. The SD of the overall laboratory mean HAdV concentrations for TCS1-9 ranged from 0.40 to 1.03 Log_{10} copies/mL. The SDs were highest for Types 7, 31 and 41. The SD of the overall laboratory means for clinical samples (representing the same HAdV types) ranged from 0.33 to 1.09 Log_{10} copies/mL. For all TCS and clinical samples inter-laboratory variation in HAdV quantification was higher than the intra-laboratory variation.

The effectiveness of candidate HAdV reference materials to harmonise viral load measurements by NAT was evaluated by 'relative potency'. For all clinical samples, apart from one of the stool samples, the SD of the overall laboratory mean was reduced when the results were expressed relative to the corresponding TCS-matrix sample. The effect of diluting the cultured virus in different sample matrices was determined by plotting the individual laboratory results for the TCS-matrix dilution series. The mean slope for each TCS-matrix sample ranged from 0.76 to 1.19.

Conclusions: The results suggest that there is variability in the quantification of different HAdV types by pan-HAdV NAT, but this would reduce through standardisation to a common reference material. A proposal to develop the 1st WHO International Standard for HAdV for NAT has been endorsed. The results of this study will be used to determine the most appropriate source material and formulation for the candidate standard. The availability of a WHO International Standard for HAdV for NAT will help to standardise these assays and enable comparison of measurements within and between different laboratories, thereby improving patient management.

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Abstract no: 132
Presentation at ESCV 2016: Poster 11

Evaluation of two algorithms for diagnosis of Epstein-Barr virus infection

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Introduction: Epstein-Barr virus (EBV) serology is mainly used to identify primary infections. Two algorithms have been proposed for diagnostic approach: the anti-VCA approach, in which the study continues if at least one of the markers, IgG or IgM, is reactive; and the anti-EBNA approach, in which the study continues only if the anti-EBNA-1 marker is negative. In our hospital we have used the full panel until now.

Since the EBV seroprevalence rate is as high as 95% amongst adults and the majority is reactive to anti-VCA IgG and anti-EBNA-1 antibodies, it is expected that the anti-VCA approach would trigger more sequels than the anti-EBNA approach, with more costs.

Objective: To evaluate if the use of the anti-EBNA and anti-VCA approaches prevent the identification of important clinical situations.

Material and methods: We have retrospectively applied both screening algorithms to all EBV serology results done in our hospital between January 2013 and December 2015 ($n = 3090$). Diagnoses obtained with both algorithms were compared with those obtained with the full panel. We have analysed the distribution of serological patterns and the clinical relevance of those patterns that would be lost with the algorithms' implementation.

Results: The anti-VCA approach would prevent the use of anti-EBNA-1 test in 3.9% of all cases. The anti-EBNA approach would prevent the use of anti-VCA IgG and IgM tests in 95.7% of all cases.

Regarding the anti-VCA approach, when we have an isolated anti-EBNA-1 pattern (suggesting a past infection), it would be classified as negative (2.94% of all cases). It will not be a problem in the investigation of a primary infection but it may be relevant in the study of diseases that we know can be associated with EBV infection (autoimmune diseases, post-transplantation lymphoproliferative disease, transplant rejection, lymphomas in the context

of inflammatory bowel disease, multiple sclerosis) or in screening for latent infection in pre-transplant patients. Our hospital has a large population of transplant patients and it seems that the savings resulting from only 3.9% of the anti-EBNA-1 tests do not justify the loss of information.

With the anti-EBNA approach, reactivity both to EBNA-1 and VCA IgM (4.43% of all cases) would be classified as past infection but, in a few patients, it could in fact be a reactivation pattern. Reactivation is of no clinical relevance in immunocompetent subjects but can cause serious complications in immunocompromised patients. In our population, immunocompromised patients (mainly infected with HIV and transplant patients) account for 13.6% of the total ammount. Therefore the use of anti-EBNA algorithm in general and the use of the full panel in immunocompromised patients would allow reducing by 82.1% the number of anti-VCA IgM and IgG tests.

Conclusion: The use of the anti-EBNA antibodies approach is acceptable with regard to primary infection diagnosis. Full panel must be used with immunocompromised patients, when reactivation is suspected. This strategy will reduce by 82.1% the number of IgM anti-VCA IgG tests without relevant clinical costs.

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Abstract no: 133
Presentation at ESCV 2016: Poster 12

Evaluation of the Veris MDx™ system for quantification of Hepatitis B DNA and Hepatitis C and HIV-1 RNA in a medium sized University Hospital

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Introduction: In the diagnosis and treatment of Hepatitis B (HBV), Hepatitis C (HCV) and HIV, it is crucial to detect and quantify viral nucleic acid. Patients on therapy are monitored continuously to out-rule relapses or reinfections (HCV) while for patients with HIV these tests are important to early on detect potential breakthroughs due to resistance development. Quantification methods are today more standardized and fast but still with no opportunity to analyze the samples with full random access. Recently the VERIS MDx™ platform from Beckman Coulter with this possibility was launched.

Objectives: To evaluate a new, random access laboratory instrument for the simultaneous detection and quantification of HBV, HCV and HIV-1.

Methods: WHO standards for HBV-DNA, HCV-RNA and HIV-1-RNA provided from the National Institute for Biological Standards and Control (NIBSC) were diluted down to the designated lowest level of detection and analyzed in triplicates on the Veris MDx™ (Beckman Coulter Inc. 250 S. Kraemer Blvd. Brea, CA U.S.A.) instrument. Plasma samples from routine laboratory testing were analyzed and compared to the routine methods used at our hospital or the referral hospital, for HBV; COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 (Roche Molecular Diagnostics, 4300 Hacienda Drive, Pleasanton, CA, USA) (Karolinska University Hospital Huddinge), for HCV; COBAS® TaqMan® HCV Test v2.0 for use with the High Pure System (Roche) (Örebro) and for HIV; Aptima HIV-1 Quant Dx Assay (Hologic Inc. 250 Campus Drive Marlborough, MA, USA) (Örebro). 55 samples for HBV, 120 samples for HCV and 60 samples for HIV have been analyzed so far. The absolute majority of samples for HCV and HIV analysis were from patients on treatment. All viral load data were analyzed as log10-transformed values.

Results: The Veris MDx™ showed good compatibility to the designated quantities of the WHO standards (except for HIV-1 where a slight over-quantification could be observed for dilutions in the higher range, i.e. >1000 copies/mL). The limits of detection assigned by the manufacturer could be confirmed. In clinical samples the Veris MDx™ showed similar results to the comparators with a correlation for quantifiable samples of 0.94 (HBV), 0.98 (HCV) and 0.98 (HIV). The Veris MDx™ showed a slightly higher sensitivity though as DNA/RNA was detected in 4 samples for HBV, 8 for HCV and 7 for HIV when the comparator method did not. The opposite was seen in 0, 0 and 6 samples respectively.

Conclusion: The Veris MDx™ for quantitative analysis of HBV, HCV and HIV nucleic acids showed good correlation to the comparator methods used in this study with a tendency of higher sensitivity for the detection of HBV and HCV. The Instrument provides an easy, fast and flexible method for quantification of RNA and DNA in plasma samples.

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Abstract no: 135
Presentation at ESCV 2016: Poster 13

Comparison of standardised and non-standardised serology assays for clinical virology diseases

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Serological assays are the bed rock of clinical virological diseases as they are relatively inexpensive, quick and lend themselves to automation. However standardisation of these diagnostic assays is incomplete as there are relatively few International Standards for clinical virology targets. Whilst we await their development how standardisation can be improved?

The Quality Control Reagents Unit (QCRU), now based at NIBSC has been producing quality control reagents for use in clinical virology testing for nearly 20 years. These QC reagents assure the quality of assays by acting as an external run control, by establishing a mean value for an assay with the reagent and then attaining this value for every assay within 2-3 standard deviations (SD) of the mean. QCRU test every batch of reagent on multiple kits and platforms, to ensure its suitability. These data allow the unit to compare the performance of a common reagent on multiple platforms. We have analysed data from three types of reagent. One where an IS exists already and 2 where development is awaited.

Analysis of data using the QCRU Rubella reagents indicate that there can be up to 2 fold variation in the reported amount of anti-Rubella antibody in the same sample. Interestingly there appeared to be less variability (<60%) closer to the cut-off of the assays than at higher concentrations. A similar variability in reporting was observed using the anti-HCV reagent on 3 different platforms where results were provided as OD/CO. The greatest variability in results reporting was observed in the reporting of anti-Mumps antibodies, where different assay platforms use different units and the figures varied between 1.2 S/CO on one platform to 728.6 EU/ml from another.

Effective comparison between antibody data generated for the same analyte on different diagnostic platforms is essential if clini-

cal practice by different groups is to be compared and best practice identified. A programme of standardisation of antibody measurement is needed for a wider range of viruses.

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Abstract no: 138
Presentation at ESCV 2016: Poster 14

External in-run controls for gastro-intestinal, respiratory infections and Zika virus that will improve assay standardisation

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Nucleic acid amplification is commonly used for rapid pathogen identification for disease diagnosis and has traditionally identified a single pathogen in a single sample. The recent development of syndromic panels that can identify multiple viruses, bacteria and parasites in a single sample has resulted in increased efficiency and also time and cost savings to the diagnostic laboratory. Whilst considerable effort is taken to validate these assays, effective standardisation is seldom undertaken that would assure the quality of data as it is generated over time and between different laboratories.

Commercially available and in house qPCR assays vary both within and between laboratories in their content and sensitivity due to varying extraction platforms, qPCR design, qPCR reagents and users. Many positive controls currently in use consist of purified plasmid DNA. This has its limitations as the construct or the preparation differs between laboratories and furthermore these plasmids do not mimic a clinical sample that has undergone extraction.

At NIBSC we have developed a multiplex in run control that contains a total of 20 viruses, bacteria and parasites to facilitate standardisation of assays for gastro-intestinal infections and a second targeting respiratory pathogens containing 15 target organisms and viruses. The pathogens were selected following consultation with multiple laboratories. The pathogens are mixed and freeze dried in a universal buffer that can be reconstituted in a matrix compatible with the end users requirements and extracted alongside the clinical samples allowing for standardisation of the extraction procedure as well as the qPCR. Greater nucleic acid stability was obtained by heating bacteria at 99 °C for 1 h when compared to ethanol treatment. The freeze drying excipient concentrations (mannitol and trehalose) were also optimised to maintain cake structure, pathogen stability and ensure commutability.

As part of NIBSC's response to the Zika virus outbreak in South America, we have developed a Zika virus reference in plasma that is being calibrated against the candidate International Standard and external in-run controls that can be reconstituted in a suitable matrix and run alongside clinical samples. These materials support monitoring of intra-laboratory variation on a daily basis and also allow standardisation of laboratory measurements of virus load.

The availability of these new external in-run controls will contribute to effective standardisation of diagnostic assays and reduce both intra- and inter laboratory variability of reported results.

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Abstract no: 144
Presentation at ESCV 2016: Poster 15

CXCL13 in patients with facial palsy caused by varicella zoster virus and Borrelia burgdorferi: A comparative study

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In an effort to improve diagnostics in central nervous system (CNS) infections, the chemokine CXCL13 has emerged as a possible diagnostic marker of Lyme neuroborreliosis (LNB). When measured in the cerebrospinal fluid (CSF), CXCL13 has shown to be significantly higher in patients with LNB compared to several other CNS infections, with the exception of cryptococcosis and neurosyphilis. Several such studies have used receiver operating characteristic (ROC) analyses, yielding a variety of suggested cut-off levels for CSF CXCL13, ranging from 61 pg/mL to 1224 pg/mL. However, patients included in previous studies presented with a variety of clinical syndromes, which raises questions on comparability. Additionally, there is no accepted reference method for CXCL13, so there may also be method-related explanations for the varying cut-offs. Facial palsy is a common manifestation of LNB, but can also be caused by varicella zoster virus (VZV) reactivation, traditionally named Ramsay Hunt syndrome (RHS). Improved diagnostics, such as VZV PCR of patient CSF, allows patients with VZV facial palsy a definite diagnose regardless of the presence of blisters associated with classical RHS. A comparison of CXCL13 in such similar patient groups has so far not been done.

28 patients with VZV facial palsy, diagnosed by detection of VZV DNA in CSF by PCR, were retrospectively identified. A total of 21 patients with facial palsy caused by LNB were included from two patient cohorts previously included in unrelated prospective studies on LNB. The median number of days between debut of facial palsy and CSF sampling was 2 (range (−)9 to 10) for VZV patients and 4 (range 1–35) for LNB patients. A control group with 52 patients without CNS infection was included. CXCL13 was measured in stored CSF samples by ELISA (R&D Systems), with a detection limit of 7.8 pg/mL.

Median CSF concentrations of CXCL13 for facial palsy caused by LNB were 1808 pg/mL (range 15–36,924), for VZV facial palsy 9 pg/mL (range <7.8 to 437); all control samples but one were below the detection limit. The differences in CXCL13 concentrations between patients with LNB facial palsy and VZV facial palsy were highly significant ($p < 0.0001$). ROC analysis-derived cut-off level of 34.5 pg/mL yielded a sensitivity of 82.6% and a specificity of 82.1%.

In this first comparative study on CXCL13 in patients with facial palsy caused by LNB and VZV, we can confirm significantly higher concentrations of CXCL13 in CSF of patients with LNB compared to patients with VZV. However, the previously proposed cut-off levels for CXCL13 would lead to unacceptably low sensitivity in our material. A cut-off at 61 pg/mL corresponds to a sensitivity of 73.9%, dropping to as low as 56% with the proposed cut-off at 1224 pg/mL. Although more than half of the patients with VZV facial palsy had

increased levels of CXCL13, the main finding of low sensitivity for diagnosing LNB using previously proposed cut-offs, is caused by the relatively low increase of CXCL13 in the LNB patients in this material. However, if only including patients where sampling was done in the first week after onset of facial palsy, the sensitivity increases to 92.9% at 61 pg/mL and 64.3% at 1224 pg/mL respectively. If CSF CXCL13 is to be used in the clinical setting, further studies on CNS infections with similar clinical presentations are needed, as the difficulties presented here requires a thorough understanding of the limitations of the analysis.

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Abstract no: 149
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Evaluation of HPV16-specific central memory T cell response in healthy subjects and patients with head–neck cancer

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Background: Growing evidences support the etiologic role of HPV16 in the onset of Oropharyngeal Head and Neck Cancer (OSCC). However HPV-related OSCC show better prognosis compared to HPV-unrelated OSCC. Recently, prophylactic vaccine has been recommended for preventing high-risk anogenital HPV infections. HPV L1 antibodies are generally weak or absent after natural infection, while high concentrations of anti-L1 antibodies are detectable about one month after the three-dose of prophylactic vaccine. It has been established that cell-mediated immune response is crucial in the control of HPV infection and correlated diseases. The principal aim of our study was to evaluate and characterize the memory HPV16-specific T-cell response in patients with OSCC.

Material and methods: PBMC from twenty-five patients with OSCC (8 females and 17 males, median age 60 years, range 40–85) were tested. Ten of 25 (40%) had biopsy-proven HPV16-related OSCC, while the remaining 15 (60%) had HPV-unrelated head–neck cancer. Additionally, long-term HPV16-specific T-cell memory was evaluated in healthy controls. Among 33 healthy subjects, six (18.2%) were males and 27 (81.8%) females (median age 24, range 21–26). Seven out of 33 (21.2%) subjects were vaccinated for high risk HPV types about 5–10 years earlier. HPV16-specific antibodies were quantified by neutralization assay and a cultured ELISPOT assay was developed to evaluate HPV16-specific central memory T-cell response. In detail, peripheral blood mononuclear cells were opportunely stimulated for 10 days in culture and restimulated for 24 h in ELISPOT plates. Pools of peptides (15 aa length with an 11 amino acid overlap) including full-length L1, E6 and E7 proteins were used as stimuli. *S. aureus* enterotoxin B (SEB) was used as positive control.

Results: A statistic difference was observed between E6-specific T-cell response in HPV16-related OSCC (median 462.0 IQR 86.0–1733.0 net spots/million PBMC* PI) and patients without HPV16-related OSCC (median 4.0 IQR 0.0–29.0 net spots/million PBMC* PI) $p = 0.0067$. A trend of significativity was observed with L1 (median 280.0 IQR 12–985 vs 1428 IQR 202–3533 net spots/million PBMC* PI; $p = 0.0631$) and E7-specific peptide pool (median 5.0 IQR 0.0–87.0 vs 60.0 IQR 28.0–607.0 net spots/million PBMC* PI; $p = 0.0951$) between the two groups of subjects. Among healthy subjects, the median antigen-specific T-cell response was significantly higher in HPV16 seropositive subjects for all antigen tested (respectively $p = 0.0124$; $p = 0.0039$; $p = 0.0179$) suggesting the persistence of an expandable long-term T-cell memory to HPV16 in healthy subjects.

Conclusion: In conclusion, we observed an increased IFN-γ producing T cells after long period stimulation with HPV16-specific antigens in patients with HPV16 related head neck cancer. The role of memory cellular response in progression and severity of cancer has to be clarify.

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Abstract no: 155
Presentation at ESCV 2016: Poster 17

Evaluation of a rapid test for the detection of Tick Borne Encephalitis (TBE) IgM in serum and CSF

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Introduction: The incidence of Tick-borne encephalitis (TBE) is increasing with 150–300 cases reported in Sweden annually. The clinical picture can be hard to differentiate from other causes of encephalitis and a rapid reliable diagnosis is therefore important. The laboratory diagnosis of TBE relies on ELISA-based testing to determine specific anti-TBE IgM and IgG in serum and in CSF. The aim of this study was to evaluate the ReaScan TBE IgM rapid test (Reagena, Toivala, Finland), a qualitative immunechromatographic lateral flow assay for the rapid detection of TBE IgM in serum and CSF.

Materials and methods: The material consisted of two blinded panels of serum and CSF. (1) 16 serum samples previously analyzed for TBE IgM and IgG using ELISA (Euroimmun) at the department of Clinical Microbiology Kronoberg County. Six of these were positive for TBE IgM, three of which had a matching CSF sample. (2) Seven (7) serum samples (6 with matching CSF samples) from patients diagnosed with TBE in Örebro County during 2015 based on IgM positivity (Immunozym) performed at the Dept of Clinical Virology, Karolinska University Hospital. All samples were analyzed using the ReaScan TBE IgM rapid test according to the manufacturer's instructions.

Results: The results obtained using ReaScan were in full concordance with the Euroimmun IgM assay for all 16 serum-samples from Kronoberg County. Of the 7 serum samples from Örebro 5 were positive for IgM with both ReaScan and Euroimmun with the remaining 2 samples being classified as Equivocal with Reascan and negative with Euroimmun. Of the 9 CSF samples tested, 2 were

positive, 2 equivocal and 5 negative. The two CSF-samples with equivocal result and three of the negative CSF samples had corresponding serum samples that were positive for TBE IgM using Reascan.

Conclusions: According to this small evaluation the ReaScan IgM rapid test seems to have a comparable performance to two commercially available ELISA assays (Euroimmun and Immunozym) for the detection of TBE IgM in serum. The two samples with equivocal Reascan-result originated from one patient on immunosuppression and one who acquired TBE despite vaccination. As others have shown the additive value of testing for IgM in CSF might be limited as only two out of nine samples here tested were positive. The Reascan TBE IgM assay seems as a valid diagnostic option for a rapid diagnosis of TBE. Additional ELISA with analysis of both IgM and IgG as well as molecular detection of TBE might be performed as confirmatory tests.

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Abstract no: 158
Presentation at ESCV 2016: Poster 18

Comparison of in-house TaqMan assay versus the Luminex Multicode technology on the ARIES platform for detection of Influenza A/B and RSV A/B

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Influenza and respiratory syncytial virus (RSV) infections are reported to cause 80–90% of viral lower respiratory tract infections being responsible for significant morbidity and mortality worldwide. It is estimated that RSV affects virtually all children by their 2nd birthday, and is the most important cause of infant lower respiratory tract infections, causing an estimated 70% of paediatric hospitalisations due to bronchiolitis. Patients with underlying health conditions are at particular risk of complicated infection with both Influenza and RSV,with the elderly and those immunosuppressed due to cancer therapies such as haematopoietic stem cell transplant (HSCT) at particular risk.

Influenza diagnosis based on clinical findings and suspicion alone has been shown to be lacking in sensitivity and specificity. Clinical differentiation between RSV and Influenza infections can be challenging due to their similar clinical presentations and as such diagnosis of infections is largely laboratory based, with timely and accurate diagnosis a cornerstone of patient management and infection control. Molecular methods have superseded conventional cell culture however their batched nature makes them inefficient for urgent testing. TaqMan PCR, though sensitive and specific, is batch tested, with time to result exceeding 3 h for urgent specimens.

The goal of this study was to evaluate the performance of the Research Use Only (RUO) Influenza and RSV assay made available by Luminex, alongside a novel, multicode based laboratory developed test (LDT) for Influenza A/B and RSV. The LDT assay adapted the routinely used TaqMan primers for use on the ARIES through modification to include IsoC at the 5′ end of the forward primer. Both the RUO and LDT methods were compared to the routinely used TaqMan PCR assay. This study also aimed to determine whether the ARIES platform and assay technology is a suitable diagnostic tool for Influenza and RSV detection in patient groups where urgent results with a highly sensitive and specific methodology are required. This is the first study to describe development of an LDT assay for Influenza/RSV on the ARIES platform.

Between January and March 2016, 219 prospective and retrospective mixed respiratory specimens were tested by TaqMan PCR and LDT assay, with 114 of these also tested with the RUO method. The RUO and LDT assays proved to be as sensitive and specific as TaqMan PCR, with much decreased operator time and time to result. The LDT assay was also able to differentiate between RSV A and RSV B. The ARIES instrument provided a simple method by which urgent specimens could be tested, with potential to allow testing 24 h per day by suitably trained staff.

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Abstract no: 162
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Antibody detection and qPCR assay for an accurate diagnosis of parvovirus B19 infection

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B19V is a virus capable of infections presenting with different courses depending on the interplay with host factors and the efficacy of the immune system response. An accurate laboratory diagnosis of B19V infection can take advantage of a multi-parametric approach, combining as far as possible the immunological detection of virus-specific antibodies to the molecular detection of viral components, mainly viral DNA

In the period September 2014–December 2015, a total of 3128 serum samples were analysed for the detection of B19V specific antibodies by a VLP-based CLIA assay (DiaSorin, Italy). Of these, 293 were also investigated by qPCR for the detection of B19V DNA and determination of the viral load (Bonvicini, 2013).

Results for antibody detection indicated, when considering a cut-off value (COV) of 1.0, 57.8% of samples positive for IgG and 11.4% for IgM (7.6% double positive); when considering a COV of 3.0, 51.7% of samples positive for IgG and 4.2% for IgM (3.1% double positive). qPCR detection of B19V DNA was employed to discriminate active infection and disease condition in a ROC analysis for IgM values. At IgM COV = 1.0, sensitivity was 0.67 and specificity 0.88, while at IgM COV = 3.0, sensitivity was 0.54 and specificity was 0.98. The low sensitivity values could be attributed to the presence of about 16–17% of PCR positive samples in the IgG positive/IgM negative sample set, indicative of persistent infections. On the other hand, the observed change in specificity in dependence of the COV was due to a more accurate discrimination of the PCR positive samples within the IgM positive sample set, from 17 to 80%.

The immunological approach to the diagnosis of B19V infection is regularly followed as a first level investigation. However, due to the characteristics of B19V infectious course and the observed difficulty in obtaining an optimal cut-off level for IgM reactivity, there remains a strong indication for molecular testing aimed to the confirmation of suspected active infections, the discrimination of persistent infections, and the follow-up of the course of documented infections.

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Abstract no: 168
Presentation at ESCV 2016: Poster 20

Comparative evaluation of the Aptima HSV 1&2 assay and a lab developed real-time PCR test for detection of HSV-1 and HSV-2 viruses

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Introduction: Herpes simplex virus 1 and 2 (HSV-1/HSV-2) cause significant morbidity in humans. Accurate diagnosis of HSV infections is important for treatment as well as counselling to reduce transmission. The Aptima Herpes Simplex Virus 1&2 Assay (AHSV, Hologic, Inc.) is an in vitro real-time nucleic acid amplification test (NAAT) for the qualitative detection and differentiation of UL42 mRNA from HSV-1 and HSV-2 on the automated Panther System. This study evaluated the clinical performance characteristics of AHSV in comparison to a lab developed real-time PCR test (LDT) targeting glycoproteins genes D and G for HSV-1 and HSV-2, respectively.

Materials and methods: Swab specimens ($n = 1249$) in viral transport medium from ocular, genital, skin and mouth lesions were submitted to the British Columbia Centre for Disease Control Public Health Laboratory for testing with the lab developed, plate-based real-time PCR test for HSV-1, HSV-2, and VZV DNA. Remnant samples were tested with the AHSV assay. Samples with discordant results were tested with a validated DNA PCR sequencing assay specific for HSV-1 and 2.

Results: A consensus result was established with both AHSV and the LDT real-time PCR assay. A total of 221 positives (17.5%; 103 HSV-1 and 118 HSV-2) were identified. For all specimens, AHSV and LDT positive agreement for HSV-1 was 82.4% and for HSV-2 was 94.4%, while negative agreement was 99.8% for HSV-1 and 99.7% for HSV-2. The kappa value for HSV-1 was 0.885 and for HSV-2 was 0.955. In 555 anogenital samples, positive agreement for HSV-1 was 89.4% and for HSV-2 was 94.1%, and negative agreement was 99.8% for HSV-1 and 99.6% for HSV-2. The kappa value for HSV-1 was 0.927 and for HSV-2 was 0.958. Most of the discordant results (29/34, 85%) were positive results obtained only by the LDT. For these, the average cycle threshold (Ct) values were 30.6 for HSV-1 and 30.7 for HSV-2, while positive samples that were in agreement had average Ct values of 20.5 for HSV-1 and 22.4 for HSV-2 ($P < 0.001$ for both targets). For samples with Ct values <30, sequencing analysis confirmed 9/11 (82%) discordant results as true positive, while for samples with Ct values >30, sequencing analysis confirmed 6/23 (26%) discordant positive results. Of the LDT positive, AHSV negative samples, 21/29 (72%) were directly adjacent to HSV positive samples with low HSV Ct values.

Conclusions: Overall, detection of HSV-1 and -2 viral mRNAs in clinical specimens using the Aptima HSV 1&2 assay showed good agreement with amplified molecular tests for HSV genomic DNA. Although the AHSV failed to detect several low positive samples identified by the LDT, some of those positives may be the result of cross contamination. The LDT used a plate-based PCR assay which can be prone to sample cross contamination. The Aptima HSV 1&2 assay is a single tube assay with very low risk of sample cross contamination that is a good alternative to LDT.

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Abstract no: 170
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Plasma and serum are suitable specimen types for quantitation of HCV RNA using real-time transcription mediated amplification or PCR

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Aims: Hepatitis C virus (HCV) RNA assays form an integral part of patient management, from confirmation of antibody results at diagnosis through to monitoring treatment efficacy. This dual purpose often means both serum and plasma samples are tested by diagnostic laboratories. The aim of this study was to evaluate performance of the new Aptima HCV Quant DX Assay (Aptima) in comparison to the Roche COBAS Ampliprep/COBAS Taqman HCV test v2.0 (CAP/CTM) using both serum and plasma sample matrices.

Method: A total of 319 surplus clinical samples were tested: 141 plasma (27 negative), 178 serum (40 negative) in parallel with Aptima and CAP/CTM assays in accordance with manufacturers' guidelines. The sample set included paired serum and plasma samples collected at the same time point from 20 patients. All samples were obtained from 225 patients received as part of routine care between July 2011 and March 2016.

Paired plasma and serum from three patients infected with HCV genotypes 1a, 3a and 4d were also used to assess assay precision. Aliquots of the plasma and serum were tested in replicates ($n = 7$) over a period of 3 days.

Three standard panels were also used: Qnostic performance panel for concordance of results; Acrometrix HCV RNA standard panel tested in triplicate to assess linearity in serum and plasma; SeraCare HCV RNA genotype performance panel was used to assess quantitation across genotypes in serum and plasma.

Results: Aptima and CAP/CTM showed excellent accuracy and linear correlation with expected results across the Acrometrix HCV RNA panel range (1.5–6.3 $\log_{10}$ IU/ml) in serum and plasma samples. In terms of precision at a nominal value of 1000 IU/ml, Aptima showed coefficients of variation up to 3.5% and 4.5% $\log_{10}$ IU/ml in plasma and serum respectively. Corresponding values for CAP/CTM were 2.1% and 2.2%. CAP/CTM reported significantly higher quantification in serum replicates but this effect was not observed in the paired plasma and serum samples of 20 patients. Within the tested clinical sample set the prevalence of genotypes 1, 2, 3 and 4 were as follows: 35%; 1%; 35%; 3% respectively and 26% were unknown. Aptima gave good quantitation across all genotypes tested, although results were generally lower than CAP/CTM in the SeraCare and Qnostics panels and closer to the Abbott Real-Time HCV results reported by SeraCare. Analysis of clinical samples showed excellent correlation (95.0%, Kappa = 0.852) between the two assays for detection of HCV RNA, and 87.5% agreement for quantitation at 15 IU/ml. Regression and Bland-Altman analysis showed a proportional bias: Aptima quantified lower than CAP/CTM closer to the lower limit of quantitation, whilst the converse was observed towards the upper limit of quantitation. The effect was similar in plasma (mean difference 0.07 $\log_{10}$ IU/ml, $R^2 = 0.9665$) and serum (mean difference 0.08 $\log_{10}$ IU/ml, $R^2 = 0.9774$).

Conclusion: CAP/CTM and the new Aptima HCV assay show excellent analytical performance in both serum and plasma. Both tests are highly accurate with equivalent sensitivity. Comparative quantitation between the two assays deviated most when close to the assay limits of quantitation. Overall, this evaluation demon-

strates the Aptima HCV Quant DX Assay is suitable for use across all genotypes with serum or plasma in clinical laboratory algorithms.

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Abstract no: 171
Presentation at ESCV 2016: Poster 22

The modular approach to respiratory syndromic testing with the fully-automated novel Panther Fusion System

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Background: Respiratory viral infections remain a leading cause of infectious diseases worldwide. Because most respiratory viruses present with similar symptoms, molecular diagnostic tools are required for rapid and accurate diagnosis to ensure appropriate patient management. Current diagnostic techniques include insensitive rapid tests, costly mega-panels, or complicated work-flow involving multiple assays. Furthermore, current methods are only approved for the diagnosis of upper respiratory tract (URT) infections but not lower respiratory tract (LRT) infections. Misdiagnosis of LRT infections of viral origin has led to overtreatment with antibiotics and increased levels of multi-drug resistance.

The Panther Fusion System and respiratory panels (IVD assays in development) address the clinical need for a flexible, modular approach to syndromic testing. The Panther Fusion Respiratory panels are qualitative multiplex RT-PCR assays which detect and differentiate multiple targets utilizing assay specific reaction mixtures. Panther Fusion offers random access capability, minimum sample handling, and short sample in-to-first result processing to identify Influenza A and B (Flu A/B), Respiratory Syncytial Virus (RSV), Parainfluenza virus 1, 2, 3, 4 (Paraflu), Adenovirus (AdV), human Metapneumovirus (hMPV), and Rhinovirus (RV). The three respiratory panels can be run from a single nasopharyngeal swab (NP) or LRT specimen (bronchoalveolar lavages, BAL; bronchial washes, BW). This study describes preliminary performance of the Panther Fusion Respiratory panels for analytical sensitivity, inclusivity, cross-reactivity, reliability in co-infection, and clinical performance.

Methods: The analytical sensitivity panel was generated by spiking viral transport medium (VTM) with various viral isolates for each target at known $TCID_{50}$ concentrations. The specificity panel was generated by spiking potential cross-reacting microorganisms into VTM at clinically relevant concentrations. For competitive interference in co-infections, viral isolates were spiked at low (0.5 log > LoD) and high (3 log > LoD) concentrations in simulated clinical matrix (SCM). Clinical performance of each viral target was compared to various on-market assays.

Results: The analytical sensitivity of the Panther Fusion assays was 10^{-2}–10^{2} $TCID_{50}$ depending on strain or serotype tested. LoDs for all three specimen types were within 0.5 log for all strains tested. No cross-reactivity between other common viruses or micro-organisms was observed. Percent agreement in reproducibility studies for all intended targets was 100%. Time-to-first result was less than 2.5 h. Concordance of clinical performance to on-market assays was high with positive and negative agreements of 93.5–100% and 96.0–100%, respectively, for all intended targets in all three assays.

Conclusions: Based on preliminary sensitivity, specificity, and clinical comparison studies, the Panther Fusion System and Panther Fusion Respiratory Panels (A/B/RSV, Paraflu, AdV/hMPV/RV) offer an unmatched combination of flexibility, throughput, and automation for respiratory viral testing in both upper and lower respiratory tract specimens.

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Abstract no: 176
Presentation at ESCV 2016: Poster 23

Performance evaluation between Seegene Anyplex II RV16 Version 1.1 and Biofire FilmArray Respiratory Panel Version 1.7 for the detection of respiratory viruses

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Background: Respiratory viral infections can cause serious complications in children, elderly and immunocompromised individuals. Rapid and precise identification of respiratory pathogens is thus critical for administering the appropriate antiviral therapy, clinical management and timely infection control measures. Recent developments in multiplex real-time polymerase chain reaction (PCR) allow for detection of multiple respiratory viruses with increased sensitivity and shorter turnaround time. Many such kit assays are currently commercially available for use in diagnostic laboratories. In this study, we evaluated the performance of two commercial assays, the Seegene Anyplex™ II RV16 and the BioMérieux BioFire RP Panel. Anyplex™ II RV16 Detection v1.1 (RV16; Seegene) is a multiplex real-time PCR using Tagging Oligonucleotide Cleavage and Extension (TOCE) technology that detects 16 respiratory viruses. The Food and Drug Administration (FDA)-cleared BioFire FilmArray® Respiratory Panel (Biofire RP Panel; BioMérieux) integrates sample preparation, amplification, detection and analysis into one simple system that requires minimal hands-on time with a total run time of around one hour. It detects 17 viruses and 3 bacteria, and has a throughput on one sample per instrument.

Methods: This study was done in two stages, retrospective and prospective, on a total of 145 specimens. For the retrospective study, 68 archived positive patient samples including bronchoalveolar lavage, nasopharyngeal swab, nasopharyngeal aspirate and sputum, which were previously characterized by the RV16 assay, were further tested on Biofire RP Panel according to the manufacturer's instructions. 22 archived CAP external quality assurance (EQA) respiratory viral specimens (2014–2015) were also ran on both assay platforms. These archived samples were stored at −80 °C before testing. Specimens with discordant results were re-extracted and re-run on the RV16 assay to rule out sample degradation. For the prospective study, 55 recently collected samples were simultaneously ran on both platforms. To resolve discordant results for influenza A/B, for which specific anti-viral treatment is available, an in-house developed PCR assay for influenza was used.

Results: The overall agreement between the two methods was high at 89% (2064/2320), calculated based on 16 viral targets. Taking RV16 as the gold standard, the overall sensitivity and specificity of Biofire RP panel was 87.2% and 97.2% respectively (95% confidence intervals, 79.1–92.5%). Biofire RP Panel appeared to be less sensitive for influenza B, adenovirus, parainfluenza 4, rhinovirus, enterovirus and metapneumovirus compared to RV16. The in-house developed assay for influenza further confirmed two discordant results of influenza B in favor of the RV16 assay. The results for 22 archived positive CAP EQA respiratory viral specimens were fully concordant between the two assays.

Conclusions: In our study, Seegene RV16 demonstrated higher sensitivity than Biofire RP Panel. Although Biofire RP Panel is simpler to run, allows for random access and comes with a shorter turnaround time, its capacity for high-throughput testing would be limited by the number of instruments available. Contrastingly, RV16 requires less effort using batch testing, which is suitable for outbreak situations when there is large influx of patient samples.

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Abstract no: 185
Presentation at ESCV 2016: Poster 24

Performance comparison of the new filmarray meningitis/encephalitis panel with routine diagnostic methods

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Background: Meningitis is the inflammatory disease of membranes that surround the brain and spinal cord. The inflammation of the brain itself is known as encephalitis. Viruses, bacteria, fungi or parasites may cause these life-threatening infections. In particular, young, elderly and immunocompromised persons are of increased risk. The incidence of acute encephalitis in Western countries is 7.4 cases per 100,000 population per year. For effective patient management and to minimize morbidity and mortality, prompt diagnosis is crucial.

The objective of this study was to evaluate the application of the Film Array (FA) Meningitis/Encephalitis panel (ME) for clinical diagnostics. Retrospective and prospective cerebrospinal fluid (CSF) clinical samples were tested in comparison with the methods routinely applied in our laboratory for the testing of pathogens in CSF specimens.

Material and methods: Residual CSF samples from patients with a high suspicion of a meningitis/encephalitis infection were included in this study. All samples were tested beforehand (stored frozen at −20 °C) or in parallel with the validated routine laboratory methods which were considered to be the reference methods (viruses: in-house real-time PCR; bacteria: conventional culture and MALDI-TOF). For this method validation, 200 μl CSF residual sample volume was tested with the FA ME panel. FA is an automated highly multiplexed closed PCR platform/system that detects 6 bacteria: *Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae*, 7 viruses: cytomegalovirus, enterovirus, herpes simplex type 1, herpes simplex type 2, human herpesvirus 6, human parechovirus, varicella zoster virus, 2 fungi: Cryptococcus neoformans/gattii.

Results: A total of 191 CSF specimens were tested. Initially, 178 FA ME results (93.2%) were consistent with the results of the reference methods. 9 of the 13 CSF specimens with discrepant results had to be excluded from the final statistical analysis since no material was left for confirmation testing. From these 13 discrepant results, 7 were prospective and 6 retrospective specimens. Only in four cases confirmation testing could be performed. Nevertheless, the analyzed data showed a final concordance between the reference methods and FA ME of 99.4%. Pathogens detected included: *Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumoniae*, enterovirus, herpes simplex type 1 and 2, human herpesvirus 6, varicella zoster virus and Cryptococcus neoformans/gatti.

Conclusions: Based on the results acquired in this study, the FA ME panel is of great value for the management of meningitis/encephalitis suspected cases where a comprehensive (14 pathogens) and fast response (in approximately 1 h) may help prevent secondary complications or even make a life-saving difference. This is particularly true for bacterial infections where an imminent antibiotic therapy is crucial for the whole recovery of the patient. As the clinical symptoms of a bacterial and viral infection for meningitis and encephalitis are often overlapping, the correct clinical diagnosis is not obvious. For the most common infectious pathogens included in the panel, this dilemma is solved by the use of FA ME.

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Abstract no: 192
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The novel ARIES platform demonstrates simplified molecular workflows and enhanced lab efficiency

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Background: Diagnostic laboratories are continuously looking for ways to maximize productivity, improve workflow, optimize staff time, and reduce the time to deliver results back to healthcare providers. In addition, there is a desire to shift from traditional testing methods to faster, more sensitive, and more cost-effective molecular methods. In this study, a time and motion study was conducted using Luminex ARIES system. ARIES is an automated, sample to answer platform that fully integrates extraction of nucleic acid from clinical samples, real-time PCR detection, data analysis, and results reporting.

Material/methods: In this study the Xpert GBS LB and ARIES HSV 1&2 assays were used as the Xpert GBS LB assay does not require any pre-processing steps and is most similar to ARIES workflow. The goal of this study was to compared hands-on time for ARIES and Cepheid GeneXpert systems, including hands-on time required to set up various numbers of samples, data entry, patient/sample information entry, and then starting the run for both systems. Two different scenarios were assessed for ARIES – (i) entering test order, sample, and assay information at the instrument using the barcode reader provided (standard workflow), and (ii) sample and cassette scanned in advance and sent to ARIES by LIS (LIS-enabled).

Results: The average time needed to load one sample was 52 s for GeneXpert and 71 s and 34 s for ARIES in standard workflow and LIS-enabled mode, respectively. For 16 samples, GeneXpert took 10 min 10 s while ARIES required 9 min 52 s (standard workflow) and 4 min 58 s (LIS-enabled mode). The reduction in hands-on time was very explicit when ARIES was configured in the LIS-enabled mode (no additional manual scanning required). A reduction in hands-on time of 45% was observed when loading 12 samples into ARIES in LIS-enabled mode as compared to GeneXpert.

Conclusions: In this time and motion study, we found that on average the set up time favored ARIES as compared to GeneXpert as less hands-on time and fewer user interactions were required. This was most pronounced if the number of samples approached six as setting up a run for a single sample was 19 s faster on GeneXpert. In general, ARIES showed reduced hands-on time, enhanced

workflow simplicity, and less chance for user error as compared to the GeneXpert system.

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Abstract no: 196
Presentation at ESCV 2016: Poster 26

Performance of a molecular diagnostic, multicode based, sample-to-answer assay for the simultaneous detection of Influenza A, B and Respiratory Syncytial Viruses

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Introduction: Rapid diagnostics is required in cases with respiratory failure for clinical decision making regarding isolation and antiviral therapy. Techniques like immune-chromatographic test (ICT) and direct immunofluorescence assay (DFA) have lower sensitivities and specificities than molecular diagnostic assays, but have the advantage of quick turnaround times and ease-of-use. Here, we evaluated the performance of an automated, easy to use, sample-to-answer system, which performs an Influenza A/B virus (fluA/B), respiratory syncytial virus (RSV) and internal control multiplex RT-PCR of 1–12 samples within 2 h.

Methods: The analytical performance of the FluA/B/RSV assay on the ARIES (Luminex), a system using multicode technology (a probe-free real-time RT-PCR method with melting curve confirmation), was evaluated using published laboratory developed automated real-time RT-PCR assays (LDA) for fluA, fluB, RSV-A and RSV-B. Genotype inclusivity of the ARIES was tested using 16 avian (H1–H16) and 33 human fluA strains, 3 fluB strains and the two RSV (A/B) strains. Specificity was assessed using 40 high positive non-fluA/fluB/RSV-viruses and analytical sensitivity was compared to LDA assays by testing 0.5 log dilution series. The clinical performance was compared to both LDA + ICT (BinaxNOW influenza A/B and RSV test) + DFA using selected (pretreated), −80 °C stored, respiratory tract samples from 2006 until 2015 (retrospective) and prospective testing of original respiratory tract samples from December 2015 onwards.

Results: All fluA, fluB and RSVA/B strains tested for analytical performance evaluation were detected and no aspecific reactions were identified. ARIES FluA/B/RSV assay was 0.5 log less sensitive for fluA, 1 log for RSV-A, 2 logs for RSV-B and 2.5 logs for fluB compared to LDA. In total, 447 samples were included in the clinical performance evaluation, of which 15.4% tested positive for fluA, 9.2% for fluB and 26.0% for RSV, (RSV-A, 13.2% and RSV-B 12.9%) in both LDA and ARIES. Confirmed discrepant results were found in 11 samples (1 fluA, 4 fluB and 6 RSV-A), which tested positive in LDA and negative in ARIES (2%, LDA Ct values 28.8–36.0), resulting in an overall clinical sensitivity and specificity of 98.6% and 100% for fluA, 91.1% and 100% for fluB and 95.1% and 100% for RSV, respectively. If compared to the DFA ($n = 217$) and ICT ($n = 119$), ARIES detected 38 (17.5%; 4 fluA, 23 fluB, 11 RSV) and 32 (26.9%; 7 fluA, 3 fluB, 22 RSV) more samples respectively, all confirmed by LDA (Ct range 14.9–35.0). In terms of robustness, 2.2% cassettes failed during operation in clinical specimen, of which 90% was an undiluted bronchio-alveolar lavage, nose wash or sputum.

Conclusion: The ARIES influenza A/B/RSV assay is a specific and rapid molecular assay. Although analytically the ARIES is less sensitive for fluB, RSV-A and RSV-B than the LDA assays, the performance in clinical samples is comparable to LDA and better than those of the established rapid assays. Other respiratory samples than throat swabs can be analyzed by the ARIES, but need to be diluted prior analysis.

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Abstract no: 200
Presentation at ESCV 2016: Poster 27

Fully automated diagnosis of MERS-CoV infection in respiratory specimen on the IdyllaTM MDx Platform

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Background: Rapid diagnosis of MERS-CoV infection is essential for the successful clinical management and isolation of MERS patients. The prototype Idylla™ MERS assay is a RT-PCR based assay which generates highly sensitive, specific results with a minimal turn-around time. Two independent PCR assays, targeting different regions in the MERS-CoV genome, are combined to detect and at the same time confirm infection with MERS-CoV. The prototype Idylla™ MERS assay is a single-use cartridge that will be run in the fully automated Idylla™ MDx Platform. The cartridge contains all reagents and is capable of processing samples without any user manipulation, minimizing the possibility of errors in setup and decreasing the risk of infection or contamination. The aim of this work was to demonstrate the performance of the prototype Idylla™ MERS assay on the Idylla™ Platform.

Methods: Performance of the prototype Idylla™ MERS assay was assessed using serial dilutions of viral culture spiked in MERS-CoV negative clinical material. The performance of the prototype Idylla™ MERS assay was compared to a conventional RT-PCR kit in combination with extraction by the NucliSENS® easyMag®. In vitro transcribed MERS-CoV RNA was used to determine the LoD of the assay and to show reproducibility. Cross-reactivity was analysed using culture and clinical specimen positive of other respiratory pathogens. Additionally, an *in-silico* analysis was performed to prove the reactivity with all available MERS-CoV sequences and to exclude any cross-reactivity with organisms present in respiratory specimen or with the human genome.

Results: The prototype Idylla™ MERS assay demonstrated high sensitivity and specificity. The analysis of MERS-CoV viral culture showed the same sensitivity with the Idylla™ MERS assay as a conventional MERS RT-PCR kit in combination with NucliSENS® easyMag® extraction. No cross-reactivity with other pathogens or the human genome was observed *in-vitro* or *in-silico*. The *in-silico* reactivity analysis showed 100% identity in 98.31% of the available sequences for the MERS screening assay and 97.07% for the confirmatory assay. The remaining sequences only showed minor mismatches and we confirmed the binding capability of our assay by using plasmids containing the mismatches.

Conclusions: The fully automated prototype Idylla™ MERS assay requires less than 2 min of hands-on time for sample handling and provides results in less than 90 min without need for experienced staff or extensive training. Due to the integrated sample preparation the handling of the infectious material is reduced to an absolute minimum. The automated sample processing and RT-PCR and data analysis will lead to a sensitive and accurate calling of any MERS-CoV positive sample. The sample-to-result format of the pro-

totype Idylla™ MERS assay offers the possibility for point-of care or centralized laboratory testing.

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Abstract no: 21
Presentation at ESCV 2016: Poster 28

Flexibility and full automation for clinical sample extraction – Performance evaluation of the new bioMérieux eMAG

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While molecular testing continues to play an increasingly important role in human diagnostics, Molecular Laboratories nowadays are confronted with numerous challenges resulting from more comprehensive test menus, consolidation of laboratory testing (including increased traceability), more stringent regulatory requirements, high throughputs and the need for rapid turnaround times. Sample preparation remains a key element in the laboratory workflow and requires processing of multiple human specimens and sample matrices, handling of different laboratory consumables, simultaneous extraction of DNA and RNA targets and coordination of eluates for downstream PCR analysis. Automation of sample extraction is a common need to master laboratory throughput and standardization whereas adaptation of automated solutions to complex workflow requirements remains a challenge. We present here results of the performance evaluation (e.g.: reproducibility, precision, LOD, carry-over, tests on different specimen types) of the new bioMerieux eMAG™ which provides full automation of sample extraction starting from primary tubes and using well established easyMAG® chemistry. Higher throughput, increased traceability and seamless integration into diagnostic laboratory's workflows have been primary design goals for this next generation platform while keeping the known flexibility of the easyMAG®.

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Abstract no: 212
Presentation at ESCV 2016: Poster 29

Evaluation of the Beckman Coulter DxN VERIS Molecular Diagnostics System (DxN VERIS) for the determination of viral load in plasma from patients infected with either HBV or HIV-1

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Background: The recently launched DxN VERIS system is a fully-automated, random-access system for the determination of viral load in infected patients. The aim of this study was to assess the performance of the VERIS HCV and HIV-1 assays against the Roche COBAS AmpliPrep/COBAS TaqMan (CAP-CTM) system and assays which is in routine use in our laboratory.

Methods: For method comparison, the plasma from 167 HBV infected patients were selected for analysis on both platforms. Similarly for HIV-1, 188 plasma samples were selected for analysis on both the DxN VERIS and Roche CAP-CTM instruments. For patient monitoring, archived plasma samples from a number of patients covering four time points were analysed on both the DxN VERIS and Roche CAP/CTM systems.

Results: For the HBV method comparison, of the 167 specimens tested, 20 samples were "not-detected" on both systems, a further 32 samples were detected but not quantified on both systems (the VERIS HBV assay linear range is 10–10^9 IU/mL, the Roche HBV v2 assay linear range is 20–1.71×10^8 IU/mL). Seven samples were quantified using the Roche HBV assay but "detected-not quantifiable" on the VERIS HBV assay. A further 11 samples were quantified using the VERIS HBV assay but were only detected and not quantifiable on the Roche HBV assay. Of the remaining 97 samples that gave results within the linear range of both assays, the correlation coefficient was determined to be 0.87 (Spearman, 95% CI 82.0–91.7). Passing-Bablok analysis illustrated an intercept value of −0.2898 with a slope of 0.939. The sample's tested ranged from 1.01–6.73 log IU/mL. Bland–Altman analysis demonstrated that there was a −0.45 log IU/mL bias on the VERIS HBV assay when compared with the Roche HBV assay. The overall profiles obtained for the patient monitoring analysis showed a good agreement between both methods. The HIV-1 data is still under analysis and will be presented later.

Conclusions: Method comparison between the VERIS HBV and Roche HBV assays demonstrated an overall concordance of 77%. There was a negative bias on the DxN VERIS system when compared with the Roche system for HBV. The VERIS HBV assay is a useful tool in the monitoring of HBV infected patients.

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Abstract no: 220
Presentation at ESCV 2016: Poster 30

Performance evaluation of the Aptima® HIV-1 Quant Dx and Aptima® HBV Quant assays on the fully automated Panther in comparison to COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 and HBV tests

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Background: Quantification of HIV-1 RNA and HBV DNA viral load plays a central role in clinical management of HIV and HBV infected patients, before and during antiviral therapy.

The Hologic Aptima® HIV-1 Quant Dx and HBV Quant are quantitative assays, being developed on the fully automated Panther system. The assay is based on real-time Transcription Mediated Amplification (TMA) technology.

Methods: *HIV*: 191 plasma samples (94 prospective and 97 retrospective) from HIV-infected patients were tested for Aptima® HIV-1 Quant Dx Assay, based on HIV viral load, as determined by routine testing using COBAS®TaqMan® HIV-1 test.

Reference panels: BioQcontrol P0043HIV-RNA, Qnostics HIV-1, HIV1 50904 and S1003 HIV-RNA DOM 046200047 were used to assess sensitivity, reproducibility and linearity.

HBV: 200 plasma or sera samples (100 prospective and 100 retrospective) from HBV-infected patients were tested for Aptima® HBV Quant Assay, based on HBV viral load, as determined by routine testing using COBAS®TaqMan® HBV test.

Reference panels: Qnostics 14038 HBV, BioQC control P0041 HBV DNA and Hologic panel were tested to assess sensitivity, reproducibility and linearity.

Cross contamination was evaluated (for both HBV and HIV) by testing 5 consecutive runs of 15 samples, composed of Hologic high and low positive control, and negative samples (Hologic diluent).

Results: *HIV:* Using a limit of quantification (LOQ) of 30 copies/ml (cp/ml) for Aptima assay, 67 specimens (1.62–6.47 log) gave quantifiable results for both assays and the Deming regression was excellent between the 2 assays: $y = 1.12x - 0.59$, $R^2 = 0.954$. 58 samples gave target not detected (TND) results for both. 66 results were discrepant at very low viral load between the 2 tests. The overall percentage of agreement was 73.3%.

- Mean difference between measured and expected values was <0.16 log cp/ml for the Qnostic positive control and <0.37 log cp/ml for serial dilution of Bio Qcontrol P0043HIV-RNA (5.40–1.70 log) tested in triplicate.
- Sensitivity assessed with serial dilution of S1003 HIV-RNA DOM 04-20047 panel was 6.25 cp/ml for 100% of 10 replicates. 3.125 cp/ml was found to be positive in 4 out of 10.

HBV: Using a LOQ of 10 IU/ml for Aptima, 132 samples gave a quantifiable result for both tests and the Deming regression was excellent between the 2 assays: $y = 0.95x - 0.07$, $R^2 = 0.945$. The overall percentage of agreement was 90%.

- Excellent reproducibility was observed with BioQcontrol with standard deviation (SD) ranging from 0.13 log at inputs below 50 IU/ml to 0.06 at inputs above 10,000 IU/ml. Results of Qnostic panels were identical to results obtained at others sites using same panels.
- Sensitivity determined with serial dilution of BioQcontrol panel was 1.56 IU/ml for 100% of 10 replicates.

No cross contamination (neither with HIV nor with HBV) was observed when testing 5 consecutive runs using negative control and High positive control samples alternately.

Conclusions: The Hologic Aptima® HIV-1 Quant Dx assay and Aptima® HBV Quant assay as performed on the fully automated Panther system gave highly comparable performance to that of Roche COBAS® TaqMan® HIV-1 v2 and HBV v2.0 assays for clinical samples. Excellent results were observed using commercially available panels indicating high sensitivity and very good reproducibility.

This system, using 0.5 ml sample input on primary samples, was easy to use and could generate 120 test results in less than four hours.

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Abstract no: 232
Presentation at ESCV 2016: Poster 31

Nucleic acid testing of blood donors – Ege University Hospital short term experience

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Nucleic acid testing (NAT) of donated blood prior to transfusion is intended to ensure that recipients receive the safest possible blood and blood products. Beginning in 1999, blood banks implemented NAT for detection of viral nucleic acids in donated blood. It is first conducted for HCV, HIV in 2003. Since 2009, blood centers have replaced the dual assay for HIV and HCV nucleic acids with a triplex assay that detects the nucleic acids of HBV in addition to HIV and HCV.

Ege University Hospital started NAT since October 2015. During the study period, a total of 17,328 donor samples were screened for serological and molecular markers of HBV, HCV and HIV. Serological screening of was performed using the Architect system (Abbott Diagnostics, Wiesbaden, Germany) (HBsAg, anti-HCV, anti-HIV I/II). Initially reactive samples were tested in duplicate using the same assay. Initial reactive blood and blood products are not used even though duplicate tests were negative. HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, anti-HBs were also tested in HBsAg negative, HBV NAT positive bloods using the Architect system (Abbott Diagnostics, Wiesbaden, Germany).

Molecular screening was performed using the Roche Cobas TaqScreen MPX v2 assay (Roche Molecular Systems, NJ, USA) on the Cobas s201 system. The MPX v2 assay is a qualitative viral multiplex test that simultaneously detects and discriminates between HBV-DNA, HCV-RNA and HIV-RNA (along with an internal control) in a single assay. Donations were screened in mini-pools of six. Mini-pool stage testing yielded either reactive or non-reactive results, but did not identify the individual infected donation(s). Reactive pool were re-tested individually to identify the agent HBV, HCV and/or HIV. Subsequent confirmatory testing was performed on the Abbott *m2000sp* and *m2000rt* (Abbott Molecular Diagnostics, USA) for the confirmation of HBV-DNA, HCV-RNA and HIV-RNA targets.

During the study period 256 donors were excluded from the study because of positive serological tests. Serological test negative 17,072 donor samples included in the NAT study. 21 pools were positive but seven of them were negative individually. HBV-DNA was confirmed with individual NAT in the remaining 14 positive pools. Twelve were anti-HBc alone, three were anti-HBc reactive together with anti-HBs and one anti-HBe together with anti-HBc. Anti-HBs values were 62, 137, 120 IU/mL. Just two samples were positive with the subsequent confirmatory test with Abbott. Their viral loads were 37 and 20 IU/mL. 14/17072 (0.08%) HBV positivity was evaluated as occult HBV.

The number of positivity is high in terms of medium endemic hepatitis B infection in Turkey. Unconfirmed positive pools remain as a problem. Hemovigilance, alternative more sensitive methods as a confirmatory test may solve unconfirmed positive pools problem.

That is for sure that NAT is reducing transfusion transmitted infections. Cost effectiveness and other algorithms may be discussed when the data accumulated.

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Abstract no: 234
Presentation at ESCV 2016: Poster 32

Qualitative detection of Zika virus RNA with the Sentosa® SA ZIKV RT-PCR test

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Introduction: Zika virus (ZIKV) is an *Aedes* mosquito-borne flavivirus that is transmitted to humans primarily through the bite of an infected mosquito. Notably, infection with ZIKV during pregnancy has been associated with fetal microcephaly and other severe birth defects as reported from Brazil in early 2015. As nucleic acid amplification technology (NAT)-based and serological assays were not readily available, the emergency response to public health threat warrants the development of diagnostics for ZIKV. The *Sentosa*® SA ZIKV RT-PCR Test is a real-time RT-PCR

based *in vitro* diagnostic test intended for qualitative detection of Zika virus RNA in clinical specimens (K_2 EDTA plasma, serum and urine) from symptomatic individuals and/or individuals who have suspected exposure to ZIKV based on epidemiological criteria. The assay workflow utilizes seamless automation from viral nucleic acid extraction (up to 24 tests) to PCR setup, followed by detection on the ABI 7500 Fast Dx system. Thus, minimizing hands-on time and human error while enabling maximum throughput with high precision.

The *Sentosa*® SA ZIKV RT-PCR Test contains reagents for reverse transcription and specific amplification of a 103 base pair (bp) fragment of the NS4A gene within the open reading frame (ORF) of the ZIKV. Additionally, the assay has a built-in positive control that acts as an external control to monitor workflow operation, a negative control to detect workflow contamination and an extraction control to confirm validity of the extraction process.

Methods: ZIKV positive samples simulated by spiking ZIKV into EDTA plasma, serum and urine were used for establishing the performance characteristics of the assay. *Sentosa*® SX101 is a liquid handling instrument that automates the extraction of samples and controls using the *Sentosa*® SX Virus Total Nucleic Acid Kit and PCR set-up using the *Sentosa*® SA ZIKV RT-PCR Test. ABI 7500 Fast Dx was used for PCR detection of ZIKV.

Results: The analytical limit of detection (LoD) resulting in >95% detection rate assessed by the workflow for *Sentosa*® SA ZIKV RT-PCR Test was determined as 6 copies/μL of viral concentration in K_2 EDTA plasma, serum and urine. The assay is reactive to both African and Asian strains of ZIKV, MR-766 and PRVABC59. The oligonucleotide sequences were aligned to 82 ZIKV sequences from NCBI using BLAST command line and revealed 100% and >95% alignment for the primers and probe, respectively. Furthermore, the assay showed no cross-reactivity to closely related flaviviruses and other viruses causing similar febrile illnesses based on wet-testing and *in silico* analysis, which would predict no potential false positive RT-PCR results. The workflow was stressed by extraction of a mixture of high positive (10,000× LoD) and negative samples to determine the chance of cross-contamination. Our data showed that the contamination rate of the workflow is 0% within and between runs. The precision of the workflow was assessed with consideration of assay lots, operators, instruments and day-to-day variability. The detection of controls and low positive samples (1.5× LoD) is highly reproducible, achieving a CV of <5% and 100% agreement.

Conclusion: We have developed a robust, minimal hands-on, high throughput and workflow-automated ZIKV NAT-based assay, which can contribute significantly to the diagnosis of ZIKV-infected patients.

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Abstract no: 246
Presentation at ESCV 2016: Poster 33

Comparative performance of new Aptima HCV Quant Dx assay with Abbott HCV Real-Time assay

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Background: It is estimated that chronic hepatitis C virus (HCV) infection affects up to 170 million people and there were 350,000 deaths due to HCV-related liver disease each year. The introduction of new direct-acting antiviral (DAA) therapies represents a relevant promise to eradicate the HCV in patients who cannot be cured with PEGilated interferon/ribavirin treatment. The use of new DAA poses new challenges for clinicians, who must accurately interpret HCV-RNA. Thus molecular HCV assay accuracy play a relevant role in the correct management of patients under therapy.

Objective: In this study, we compare the concordance of absolute quantification of ABBOTT HCV RealT*ime* assay (ABBOTT HCV-Abbott HCV, LOQ = 12 IU/ml) and Aptima HCV Quant Dx assay (Aptima HCV-Aptima HCV, LOQ = 10 IU/ml) by using diluted HCV WHO standard samples as well as by testing clinical samples of chronically HCV-infected patients attending INMI L. Spallanzani IRCCS Hospital.

Materials and methods: Serial dilutions were prepared from 5th WHO standard (NIBSC code 14/150, genotype 1a) to achieve nominal concentration corresponding to 2000, 1000, 500, 250, 125, 50, 25,12.5, 6.25 IU/ml. Ten replicates (2000; 1000;500; 250; 125; 12.5; 6.25 IU/ml conc.) or 20 replicates (50; 25 IU/ml conc.) were tested. % CV values based on long-transformed results were calculated for each dilution and both platform. In the second approach, 117 prospective and 178 retrospective clinical samples, were tested by side-by side in the two assays.

Results: A good correlation were observed between the expected and observed results obtained both with Aptima HCV ($r = 0.998$) and Abbott HCV ($r = 0.9011$). Abbott HCV showed a lower % CV in comparison to Aptima HCV at 2000 (0.84 vs 2.37); 125 (4.08 vs 7.15), at 12.5 IU/ml (5.30 vs 7.36), at 50 IU/ml (3.36 vs 6.36), and at 6.25 IU/ml (14.26 vs 15.62). % CV variation was lower in Aptima HCV at the following WHO standard concentration: 1000 IU/ml (2.76 vs 2.97), 500 IU/ml (2.54 vs 2.71), 250 IU/ml (2.54 vs 2.71), 12.5 IU/ml (12.28 vs 12.76). At HCV RNA concentrations higher than 125 IU/ml, Abbott HCV tended to underestimate HCV RNA level. Although detecting all replicates, Aptima HCV quantified 9/10 samples at 12.5 IU/ml and 6/10 samples at 6.25 IU/ml. Among clinical samples, we observed 23 discrepant results (23/117, 19.7%). They were retested with COBAS Ampliprep/COBAS TaqMan v.2 (Roche). Six samples resulted "not-detected" with Aptima HCV and Roche and detected >12 IU/ml or quantifiable by Abbott HCV. Two samples were detected <10 IU/ml by Aptima HCV and "not detected" by Abbott HCV and Roche assays. The other samples showed variable HCV RNA values in the 3 considered assays.

Conclusion: Overall evaluation and comparison of the Aptima HCV and Abbott HCV commercially HCV RNA assays revealed comparable precision, quantification level, and detection rates. Consequently, HCV RNA monitoring during therapy, monitored with both platform, can be considered comparable for treatment decisions.

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Abstract no: 254
Presentation at ESCV 2016: Poster 34

Evaluation of the Aptima HIV-1 Quant Dx assay on a wide panel of HIV-1 strains

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Background: Plasma viral load (pVL) is usually used to monitor HIV infected patients, to measure viral replication level and virological response to treatment. Recently, the new system Panther (Hologic) and the Aptima HIV-1 Quant Dx viral load assay have been commercialized. This new generation of automaton gives faster results due to random access loading of any sample at any time and because all steps are performed in the instrument. The assay is based on amplification of two targets (pol and LTR) using real-time TMA. Before being widely adopted, the practicability of new commercial systems must be evaluated as well as the performances of their assays for detecting and quantifying the wide genetic diversity of HIV-1 strains.

Methods: Aptima HIV-1 Quant Dx on Panther system (Hologic) was compared to RealTime HIV-1 on m2000SP/m2000RT system (Abbott Molecular); the Lower Limit Of Quantification was 30 cp/mL and 40 cp/mL for Aptima and RealTime respectively; both assays were linear to 10 million cp/mL.

90 HIV-1 group M selected plasma samples (15 subtype B, 75 non-B subtype) previously quantified with Real-Time, 201 plasma prospectively collected, and 72 culture supernatants (49 HIV-1/M, 2 HIV-1/N, 20 HIV-1/O, 1 HIV-1/P) were compared. Data analysis was performed using Bland- Altman graph and Passing Bablok regression.

Results: Five of the 90 selected plasma were detected with Aptima (pVL between 43 and 137 cp/ml with Real-Time). Twelve (1 B and 11 non-B) of the 85 quantified samples by both assays had higher pVL (+0.51 to +0.9 Log) with Aptima, with a mean of differences of 0.22 Log.

112 of the 201 prospective plasma were undetectable, 17 detected and 16 quantified by both assays. 41 (20.4%) were detected with Aptima and undetectable with Real-Time and 9 (4.5%) the opposite. 3 (1.5%) were quantified with Aptima and detected with Real-Time (pVL between 40 and 204 cp/ml) and 3 the opposite (pVL between 45 and 53 cp/ml). All HIV-1 group M supernatants were quantified; higher values were obtained with Aptima (mean of differences = 0.35 Log) and 10 samples had differences between 0.51 and 0.98 Log in favor of Hologic and one (0.51 Log) in favor of Abbott. Five group O supernatants were detected, but not quantified by Aptima (pVL between 3.5 and 3.98 Log with Real-Time), two were underquantified with Real-Time (−0.53 and −2.88). The 2 groups N and the group P gave higher values with Aptima (+1.29 to +1.72 Log).

Conclusion: Despite the major underquantification of 5 HIV-1 group O strains, performances of the Aptima assay on a wide panel of HIV-1 strains are very good with globally a pVL mean higher as well as more samples detected, than with the Real-Time assay.

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Abstract no: 264
Presentation at ESCV 2016: Poster 35

CE-IVD validation of EBV ELITe MGB® assay in combination with ELITe InGenius™, an innovative sample-to-result solution for in vitro diagnostic

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Background: The "EBV ELITe MGB® Kit" is a qualitative and quantitative nucleic acids amplification assay for the detection and quantification of Epstein-Barr human herpetic Virus (EBV). The validation study was performed, on whole blood and plasma samples, in combination with ELITe InGenius™, the first fully automated sample-to-result solution introduced with a comprehensive quantitative transplant pathogen monitoring menu.

Material/methods: EBV ELITe MGB assay (ELITechGroup Molecular Diagnostics) is a Real-Time PCR assay based on MGB technology. ELITe InGenius™ (ELITechGroup Molecular Diagnostics) automatically performs nucleic acid extraction, PCR set-up, Real-Time PCR amplification and results analysis. The evaluation of the performances was designed according to European requirements for CE-IVD marking. The tests included: (1) verification studies of the PCR performance to assess efficiency, linearity, precision, accuracy, repeatability, reproducibility and sensitivity for the target and internal control, and (2) system performance verification studies using four certified reference material panels (Qnostics Ltd. and Acrometrix). The conversion factor to International Unit was calculated using a panel of dilutions of calibrated reference material ("1st WHO International Standard for Epstein-Barr Virus (EBV) for Nucleic Acid Amplification Techniques", NIBSC code 09/260, UK). The clinical study included evaluation of diagnostic sensitivity and specificity that were assessed by testing positive EBV clinical samples and negative donor samples for each sample matrix.

Results: The PCR analytical sensitivity was verified at 10 copies/reaction. All certified references samples were correctly detected and correctly quantified in international unit with a titer within the expected value ±0.5 Log, except one educational sample close to the lower limit of quantification of the test. The clinical sensitivity was respectively 100% (30/30) and 100% (47/47) for whole blood and plasma samples. The clinical specificity was respectively 90.6% (29/32) and 98.4% (60/61) for whole blood and plasma samples. When used in association to ELITe InGenius, EBV ELITe MGB assay meets all the verification and validation criteria.

Conclusions: The results obtained support the CE-IVD marking of EBV ELITe MGB kit in combination with the ELITe InGenius™ system for the detection and the quantification of EBV DNA in whole blood and plasma samples and for the diagnosis and monitoring of EBV infections especially in transplant patients.

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Abstract no: 267
Presentation at ESCV 2016: Poster 36

Comparison of respiratory and Meningitis/Encephalitis viruses detected by FilmArray® multiplex PCR versus real-time PCR

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Introduction: Fast and reliable pathogen detection is important for adequate management of infections. Although real-time PCR (rtPCR) is usually the most sensitive method for direct pathogen detection, it requires experienced technicians, includes several working steps and has a turnaround time of multiple hours. Therefore this method is not ideal for emergency diagnostics. The FDA cleared, fully automated sample to answer, FilmArray® (FA) multiplex PCR system (BioFire/bioMérieux) detects a broad spectrum of pathogens in ~70 min. To optimize our diagnostic services during weekends and off-peak times, we compared the FA Respiratory Panel (RP) and FA Meningitis/Encephalitis (ME) Panel to our routinely used rtPCR assay. The FA panels detect 20 respiratory pathogens (17 viruses, 3 bacteria) in nasopharyngeal swabs (NPS) and 14 M/E pathogens (7 viruses, 6 bacteria, *Cryptococcus neoformans/gattii*) in cerebrospinal fluids (CSF).

Materials and methods: With FA we tested 84 retrospective samples (23 NPS, 29 broncheoalveolar lavages [BALs], 32 CSF) and 60 prospectively collected NPS that required urgent testing during the 2015/2016 flu season by FA and rtPCR. FA sample input volume was 300 ml for RP and 200 ml for ME. Commercial RP and ME quality control panels (MMQC Inc., Scarborough, USA), containing samples positive and negative for each analyte detected by the FA panels, were tested multiple times. For rtPCR, nucleic acids were extracted from 220 ml of sample and eluted in 55 ml using NucliSENS easyMAG (bioMérieux). Respiratory viruses were analyzed by real-time PCR using a combination of 7 duplex Respiratory Multi Well System r-gene™ (RG) assays (influenza A/B, RSV/hMPV, HRV&EV/cell control, ADV/HBoV, HCoV/HPIV1-4) (Argene/bioMerieux), according to manufacturer's instructions. Additionally, we expanded FA RP testing to include (BALs), by implementing one additional sample preparation step. CSF was analyzed for virus using laboratory developed tests (LDTs) certified by the Swiss authorities.

Results: RP and ME quality control panel results were 100% concordant with expected results. For all NPS, both tests, FA RP and RG, identified one or more viruses in 45/83 (54.2%) samples. FA RP and RG results correlated for 42/48 viruses detected (87.5%). FA RP detected an additional 3 HRV/EV and RG detected additionally 1 FluA, 1 ADV and 1 HRV/EV. Positive percent agreement (PPA) between RG (laboratory standard) and FA RP for NPS was 93.3% and negative percent agreement (NPA) was 92.7%. Overall correlation was 93.2%. Results from BALs yielded 92% PPA, 93.1% NPA and overall correlation of 92.4%. For FA ME testing, 31/33 CSF samples had identical FA ME and LDT results with an overall correlation of 94.4%. FA ME did not detect 2 parechovirus low level LDT positive samples (Ct 36.3 and 37.0). Using LDTs as the laboratory standard, FA ME PPA and NPA were 93.9% and 100%, respectively.

Conclusion: Results obtained with the FilmArray® RP and ME panels were highly concordant with our currently used diagnostic methods, demonstrating excellent performance. The simplicity of the FilmArray® system, requiring less than 5 min of hands-on time, easy to read reports, and low sample volume allows for testing during off shifts and when urgent results are required. The comprehensiveness of the FilmArray® panels is ideal for diagnosing clinical syndromes where there are many potential causes.

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Abstract no: 273
Presentation at ESCV 2016: Poster 37

Cross-contamination and carry-over study results obtained with ELITe InGenius, a new sample-to-result solution for in vitro diagnostics

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Background: ELITe InGenius™ (ELITechGroup Molecular Diagnostics) is a fully automated sample-to-result solution, designed for the *in vitro* molecular diagnostics and monitoring of infectious diseases. The system combines on a single platform: sample processing (extraction and purification), PCR set-up, real-time amplification and detection of multiple parameters for qualitative and quantitative analysis, and result interpretation. Absence of cross-contamination and cross-over was evaluated within robustness study.

Material/methods: ELITe InGenius features a universal extraction in a unitary cassette based format (ELITe InGenius SP200) and multiple and independent Real-Time PCR with mixed parameters including CE-IVD Real-Time PCR assays (ELITe MGB line) and Laboratory Developed Tests. One to twelve patient samples can be processed in 12 parallel tracks within one run. Cross-contamination and cross-over study protocols were designed in accordance with FDA Draft Guidance for Industry and Food and Drug Administration Staff Establishing the Performance Characteristics of Nucleic Acid-Based In vitro Diagnostic Devices. The tests to evaluate the rate of false positive included: (1) 30 high positive MRSA samples (10^7 CFU/ml, prepared by dilution of MRSA BAA-1720 strain) tested alternating to 30 negative samples within five 12-sample runs with MRSA/SA ELITe MGB kit; (2) negative MRSA samples ($n = 50$) tested within five runs along with 1 positive and 1 negative MRSA/SA controls per run with MRSA/SA ELITe MGB kit, and (3) 30 high CMV positive samples (10^4 IU/ml, prepared by dilution of the "1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques") tested alternating to 30 negative samples within five 12-sample runs with CMV ELITe MGB kit.

All samples were tested carrying out the whole analysis procedure: extraction, amplification, detection and result interpretation with ELITe InGenius™ in combination with ELITechGroup reagents.

Results: All negative and all positive MRSA and CMV samples were correctly identified by the system. 100% of concordance with the expected results was obtained for all the samples tested.

Conclusions: The results obtained demonstrated the total absence of carry-over and cross-contamination of the system even when high positive samples were tested. They confirm the robustness and the reliability of ELITe InGenius for *in vitro* Molecular Diagnostics testing.

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Abstract no: 279
Presentation at ESCV 2016: Poster 38

Validation of ELITe InGenius™, a flexible sample-to-result solution, for viral meningitis and encephalitis testing

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Background: ELITe InGenius™ (ELITechGroup Molecular Diagnostics) is a new and fully automated cassette based sample-to-result solution combining a universal extraction and independently controlled real-time PCR thermal cyclers. The system was CE-IVD validated for the rapid diagnostic and the monitoring of viral meningitis based on the detection of Enterovirus, HSV1, HSV2 and VZV with ELITe MGB assays in cerebrospinal fluid (CSF) patient samples. This study describes the performance verification and validation of these 4 assays on CSF samples and its advantages in terms of workflow.

Material/methods: ENTEROVIRUS ELITe MGB® Kit, HSV1 ELITe MGB® Kit, HSV2 ELITe MGB® Kit and VZV ELITe MGB® Kit (ELITechGroup Molecular Diagnostics) are real-time PCR assays based on MGB technology. ENTEROVIRUS ELITe MGB® Kit is a one-step assay: reverse transcription of the viral RNA and Real-Time amplification are performed in the same reaction. The four assays were validated with ELITe InGenius™ using a universal extraction process in unitary cassette based format (ELITe InGenius™ SP 200) validated for both viral RNA and DNA extraction. The CSF volume required was 200 μL. The complete volume was processed by the system with an internal control to check the integrity of the full process. The extracted nucleic acids were eluted in 100 μL in a specific collection tube. 20 μL of extracted samples were then used for each DNA based assays (HSV1, HSV2, VZV ELITe MGB assays) and 10 μL for the Enterovirus ELITe MGB assay. The validation study included: (1) analytical studies to verify the PCR performances: efficiency, linearity, accuracy, repeatability, reproducibility, sensitivity; and (2) clinical study to assess the diagnostic sensitivity and specificity by testing with each assays: positive clinical and contrived CSF samples ($n = 20$) and negative CSF samples ($n = 22$).

Results: The four real-time PCR assays passed the performance acceptance criteria established for both analytical studies and clinical studies on CSF samples. The global diagnostic sensitivity obtained with the four ELITe MGB kits was 100% (80/80) and the diagnostic specificity was 100% (88/88) on the CSF samples. In terms of workflow, the storage of the extracted nucleic acids in a dedicated tube and the independently controlled unitary thermal cyclers enable the laboratory to perform multiple PCR in parallel from one single CSF extracted sample, even with different thermal profiles, or to perform the PCR reactions in separate runs with the possibility to store the remaining eluate for retesting or archiving.

Conclusions: The results of the studies support the CE-IVD marking of ELITe InGenius™ in combination with Enterovirus, HSV1, HSV2 and VZV ELITe MGB assays for viral meningitis and encephalitis testing. The unique design of ELITe InGenius™ enables the laboratory to use single assay or to create custom panels combining the four parameters according to their specific needs simply on demand. The resulting flexibility associated to a short hands-on time could contribute to improve the laboratory workflow for viral meningitis testing. ELITe InGenius™ is the first sample-to-result solution with a CE-IVD quantitative menu for viral meningitis testing.

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Abstract no: 288
Presentation at ESCV 2016: Poster 39

Comparing performance of NxTAG RPP, xTAG RVP Fast v2 and FilmArray RP for detecting respiratory pathogens in nasopharyngeal aspirates and swine/avian origin influenza A in culture isolates

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Introduction: Rapid and accurate identification of the causative pathogens of respiratory tract infections including influenza can help guide treatment decisions with specific antiviral therapy, implementation of infection control measure, possibly reducing the length of hospital stay and associated healthcare costs.

Objective: This study is to compare the performance of NxTAG RPP (CE-IVD) with xTAG RVP Fast v2 IVD and FilmArray for detecting respiratory pathogens in nasopharyngeal aspirates, and swine/avian origin influenza A in culture

Materials and methods: Seventy-one NPA samples collected from Queen Mary Hospital patients suspected with respiratory viral infections together with two swine and five avian origin influenza A culture isolates: H1N1pdm09, H3N2 variant, H2N2, H5N1, H5N6, H7N9 and H9N2 were recruited for this evaluation. Testing will be performed according to the respective product inserts for NxTAG RPP, xTAG RVP FASTv2 and FilmArray AP.

Results: The sensitivity, specificity, positive predictive value and negative predictive value of NxTAG RPP, xTAG RVP and FilmArray AP are 97.1%, 99.7%, 95.2% and 99.8%; 96.9%, 99.9%, 99.0% and 99.7%; 85.3%, 99.9%, 98.8% and 98.9% respectively. When NxTAG RPP was compared with xTAG RVP and FilmArray RP, the concordance of positive and negative results, Kappa is 0.95 (95% CI 0.91–0.98) and 0.92 (95% CI 0.87–0.98) respectively, while xTAG RVP compared with FilmArray RP, Kappa is 0.90 (95% CI 0.85–0.95). All influenza A subtype isolates were detected by matrix gene and the mean of analytic sensitivity of NxTAG RPP, xTAG RVP and FilmArray RP were 0.3, 12.3 and 0.5 of $TCID_{50}$ respectively. The genotyping gene remains negative except H1N1pdm2009 isolate. H3N2 variant was subtyped as seasonal H3N2 by NxTAG RPP.

Conclusion: In this study, NxTAG RPP, xTAG RVP and FilmArray AP for detecting respiratory pathogens have high concordance results between them. NxTAG RPP was the most sensitive for detection of respiratory pathogens than NxTAG RVP and FilmArray RP. Despite NxTAG RVP gives incorrect subtyping of H3N2 variant, it is still the most sensitive assay for detection of avian or swine origin Influenza A that threaten human life.

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Abstract no: 292
Presentation at ESCV 2016: Poster 40

Comparison of the recently launched Hologic Aptima HBV Quant assay with the established Abbott RealTime HBV assay in viral load measurement

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Background: Hologic's Aptima HBV Quant assay is a HBV DNA quantitative assay based on real-time Transcription Mediated Amplification (TMA) that runs on the fully automated Panther system with random access. A comparison with the Abbott m2000 RealTime assay was performed. Special focus with clinical samples was put on reproducibility, linearity, sensitivity and performance in different genotypes.

Methods: Fresh ($n = 450$), frozen ($n = 178$; 28 with known genotype) and diluted ($n = 618$) patient samples spread over the clinical relevant range were tested. Analytical sensitivity of the Aptima assay was assessed using dilutions of the AcroMetrix HBV standard (SKU950150) run in replicates of 10/dilution. Linearity of both assays was tested by dilution series of patient samples with HBV genotypes A-F from 8.0 to 2.0 log IU/mL in replicates of 3. Intra-assay variation was calculated by testing 30 replicates of a clinical sample in three dilution steps of genotypes A, D and one unspecified in both systems. Inter-assay variation for the Hologic Aptima system was assessed testing replicates of clinical samples with genotype A, D and one unspecified in three dilution steps on 20 different days. Discrepant samples with a difference in viral load greater 0.5 log IU/ml were retested with the Roche CAP/CTM HBV assay.

Results: Aptima HBV Quant assay showed excellent performance in high throughput routine. The calculated lower limit of detection (LLOD) using the Acrometrix standard was 2.02 IU/mL (plasma, package insert: 5.58 IU/ml). Regression models demonstrated high concordance between the two assays for all genotypes. In the correlation analyses for all tested samples the slope was 0.97 with an intercept of 0.17 and R^2 of 0.94. Bland Altman plots (Aptima minus RealTime) showed a mean difference of 0.045 with no change in bias over the complete range from 10 IU/ml up to 650.000.000 IU/ml. Linearity was proofed by serial dilution from 8 log IU/mL to 2 log IU/mL showing no difference between the two assays. Intra- and inter-assay variation was low and comparable to RealTime with intra-assay %CV ranging from 1.9% for samples with a viral load of 3.0 log IU/mL to 16.6% with 1.3 log IU/mL. 44 samples with a difference of greater than 0.5 log IU/ml were retested. Most of the discrepant samples showed higher values in the Aptima assay as compared to the Abbott assay. This was supported by the Roche CAP/CTM, which also showed higher values than the Abbott assay, though not as high as the Aptima assay.

Conclusion: The Aptima HBV Quant assay showed good correlation with Abbott RealTime with the same high sensitivity, linearity and accuracy for all tested HBV genotypes. In this large comparison study only a small amount of samples showed discrepant results. These were mainly in the low to intermediate viral load range and showed a higher quantification in the Aptima assay, what was supported by the results of retesting those samples with the Roche CAP/CTM assay. With random access and time to first result of about 150 min this assay is a major improvement in the viral load monitoring of HBV infection.

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Comparison of two quantitative detection assays of cytomegalovirus DNA

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Background: Cytomegalovirus (CMV) represents the major infectious cause of birth defects, as well as an important pathogen for immune-compromised individuals. Quantitative DNA detection of CMV is critical in the management of transplant patients. In this study, we compared the performance of two commercially available CMV quantitative PCR assay in plasma for the diagnosis of CMV infection.

Materials and methods: Two commercially available assays: RealStar® CMV PCR Kit 1.0 (altona Diagnostics) and CMV real time PCR Kit with extraction control (Quidel) were performed according to the manufacturers' instruction on (1) The 1st WHO standard for CMV (09/162) with 10 folds dilution; (2) QCMD panel 2015; (3) Thirteen plasma samples collected from Department of Clinical Microbiology, Vejle Hospital, Denmark with positive CMV PCR.

RealStar® CMV PCR Kit 1.0 uses four concentrations for the standard curve, while the Quidel CMV kit uses only three concentrations for the standard curve.

Results: The 1st WHO standard: The detection limit for RealStar® CMV PCR Kit 1.0 is 5×102, while for Quidel CMV kit was 5×103. Most of the results from Quidel CMV kit measured CMV more than 10 times higher than the true value, while quantitation using RealStar® CMV PCR Kit 1.0 is very close to the true value (Table 1).

QCMD panel 2015: There were ten samples from two distributions. Results from RealStar® CMV PCR Kit 1.0 were very close to the expected QCMD results. Results from Quidel CMV kit were all around one log10 IU/ml higher than the QCMD results (Table 2[b]).

Table 1

1st WHO standard concentration	Altona CT value	Altona IU/ml	Quidel CT value	Quidel IU/ml
5×10^6	22.64	8.2×10^6	24.18	1.5×10^8
5×10^5	26.11	7.2×10^5	27.76	1.1×10^7
5×10^4	30.57	3.1×10^4	32.19	4.0×10^5
5×10^3	33.82	3.2×10^3	35.98	2.4×10^4
5×10^2	37.50	2.4×10^2	0	0
5×10^1	0	0	0	0
5×10^0	0	0	0	0

Table 2

Sample ID	Altona IU/ml	Altona Log_{10}IU/ml	Quiel IU/ml	Quidel Log_{10}IU/ml	QCMD Log_{10}IU/ml
CMV15C1-1	0	0	0	0	2.407
CMV15C1-2	7863	3.896	937745	4.97	3.916
CMV15C1-3	8217	3.915	138175	5.14	3.916
CMV15C1-4	1164	3.066	9358	3.97	2.986
CMV15C1-5	10101	4.004	59300	4.77	3.873
CMV15C2-1	219	2.34	10135	4.01	2.707
CMV15C2-2	0	0	0	0	0
CMV15C2-3	727	2.862	11483	4.06	3.087
CMV15C2-4	110	2.041	0	0	2.151
CMV15C2-5	7959	3.901	124725	5.1	4.107

Clinical positive samples: The PCR efficiency using RealStar® CMV PCR Kit 1.0 was in the range 102.2–105.4, while the PCR efficiency using Quidel CMV kit was in the range 111.6–124.2. Six of the 13 samples showed ≥10 times higher viral load using Quidel CMV kit than using RealStar® CMV PCR Kit 1.0.

Conclusions: Overall, both CMV assays performed well at our laboratory. Viral load measured by RealStar® CMV PCR Kit 1.0 were very close to the expected results from both QCMD panel and 1st WHO standard, while viral load measured by Quidel CMV kit were about one Log 10 IU/ml higher than expected. The same was seen for the clinical samples, where Quidel CMV kit resulted in a higher viral load compared to RealStar® CMV PCR Kit 1.0 for almost half the samples. Based on the comparison it appeared that using Quidel CMV kit would result in a higher level of viral load compared to RealStar® CMV PCR Kit 1.0. This could potentially influence treatment of patients.

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Abstract no: 304
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Performance of the IDS-iSYS "walk away" immunoassay system for the assessment of immunity to rubella virus and cytomegalovirus

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Background: Reliable and easy screening of IgG and IgM antibodies against Rubella virus (RV) and Cytomegalovirus (CMV) is of crucial importance in pregnant women in order to differentiate absence of infection, past infection, primary infection or reinfection. Here we report the performance of a new commercial automated immunoassay system, IDS-iSYS, for the assessment of immunity to RV and CMV on a large panel of samples.

Material and methods: Two panels of 168 sera (162 patients) and 350 sera (331 patients) were retrospectively selected from our university hospital routine screening for RV and CMV serology, respectively.

The immunoassays routinely used in our laboratory for the detection of IgG (± IgG avidity) and IgM antibodies against RV and CMV (Enzygnost® Immunoassay, Siemens Healthcare Diagnostics; Ela test PKS® MedacDiagnostics and Vidas® assay bioMerieux) were used as "reference tests" to classify the samples as (i) seronegative (IgG and IgM negative); (ii) past infection (IgM neg, IgG pos with high IgG avidity); primary infection (IgM pos and IgG pos with low IgG avidity) or reinfection or reactivation (IgM pos and IgG pos with high IgG avidity

The determination of RV and CMV IgG avidity with the IDS-iSYS system was also compared to the IgG avidity assessed with the Vidas assay. Sensitivity (Sens.), specificity (Spec.) and concordance (Conc.) of the IDS-iSYS parameters were compared to the reference tests.

Results: RV panel-sera: 50 seronegative, 67 past infections, 36 primary infections, 1 reinfection. The IDS-iSYS IgG Sens., Spec. and Conc. compared to the Enzygnost IgG assay were 97.4% (95% IC:96.4–98.9), 100% (95% IC:92.9–100) and 96.7 (95% IC:92.4–98.6), respectively. The IDS-iSYS IgM Sens., Spec. and Conc. compared to Vidas IgM assay were 100% (95%: CI 89.2–100), 100% (95%: CI 97.1–100) and 100% (95% CI: 97.6–100). The IDS-iSYS IgG avidity assay was tested on 24 and 52 sera with respectively a low or high avidity with the Vidas avidity assay and the overall Conc. was 94.8%. The specificity of the IDS-iSYS IgG avidity assay to exclude a primary infection of less than 2 months was 100%.

CMV panel-sera: 116 seronegative, 130 past infections, 101 primary infections. The IDS-iSYS IgG Sens., Spec. and Conc. compared with the Enzygnost IgG assay were 98.7(95% CI: 96.2–99.6), 99.2% (95% CI: 95.2–99.9) and 98.9 (95% CI: 97–99.6), respectively. The IDS-iSYS IgM Sens., Spec. and Conc. were compared to the ELAT Test PKS Medac IgM assay as reference test. The Spec. was 100% (95%: CI 96.7–100) both for the seronegative panel and the past infection panel (CI 95%: 98.4–100). The Sens was 94.3 (95%:CI 87.3–97.6). The Conc was 95.4% (CI 95%: 92.6–97.2). The IDS-iSYS IgG avidity assay was tested on 96 and 107 sera with respectively a low or high avidity with the Vidas avidity assay and the overall Conc. was 92.6% (95%: CI 88–95.5). The specificity of the IDS-iSYS IgG avidity assay to exclude a primary infection of less than 3 months was 100%

Conclusion: The IDS-iSYS is a real "Walk-Away System"; easy to use, fast and secure and appears to offer new reliable commercial immunoassays for the detection of IgG and IgM antibodies against Rubella virus and Cytomegalovirus.

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Abstract no: 31
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Characterization of oseltamivir-resistant population dynamics in immunosuppressed patients with prolonged excretion using ddPCR platform and comparison with deep sequencing analysis

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Introduction: The H275Y mutation in neuraminidase (NA) is the most frequently encountered mutation responsible for oseltamivir-resistance in A(H1N1) influenza viruses (IV). Digital Droplets PCR (ddPCR) is a rising method to explore single nucleotide polymorphism (SNP). ddPCR is known to have higher sensitivity than real-time PCR (qPCR) and ability to obtain absolute quantifications for subpopulations. After comparison of ddPCR, qPCR and deep sequencing (NGS) performances, we explored the resistant subpopulation kinetics for two immunocompromised patients with sustained shedding of A(H1N1)pdm09.

Methods: Overall 90 samples were analysed by two PCR technics using same primers and probes. (i) qPCR was performed using ABI 7500 platform (Applied Biosystem, USA). Results were analysed using SDS software. (ii) ddPCR assay was carried out according to manufacturer's instructions using the QX100 ddPCR platform (Biorad laboratories, USA). ddPCR results were analysed by Quantasoft® software. We strengthened our results by a NGS assay using PGM platform (Lifetechnologies, USA). Reads were analysed using SamTools software and pileup files were analysed to evaluate the proportion of each variant of interest. Discrimination performances and sensitivity of the ddPCR assay were evaluated on mixes of wild type (WT) H275-NA and mutated Y275-NA-coding segments at different concentrations. Then, we evaluated mutation frequency

in 10 nasopharyngeal swabs obtained from two immunocompromised patients ($n_1 = 7$ and $n_2 = 3$) with extended A(H1N1)pdm09 viral shedding.

Results: High correlations (using Spearman correlation test) were observed between qPCR and NGS, ($r^2 = 0.9187$, $p < 0.01$), and between ddPCR and NGS ($r^2 = 0.9132$, $p < 0.01$). ddPCR demonstrated higher performances than qPCR, using NGS assay as a gold standard. ddPCR was able to detect 1.5–2% of oseltamivir-resistant viruses in a WT IV population and 3–4% of WT IV in an oseltamivir-resistant population (variation coefficient <10%). In the clinical specimens of the first patient, the oseltamivir-resistant IV population reach 56% 14 days after the beginning of oseltamivir treatment. The treatment was then stopped during 22 days and resistant IV population dropped to be below quantification level by qPCR and ddPCR (<1.5%) Oseltamivir treatment was re-introduced and oseltamivir-resistant IV population rocketed up to 96% in 5 days before lethal complications. For the second patient, oseltamivir-resistant IV populations reached 22%, 9 days after treatment onset, on the last specimen collected before death.

Discussion: Use of new molecular tools can improve accuracy and speed of diagnosis especially for infectious diseases when therapeutic resistance may lead to severe complications. Our study showed that ddPCR is cheaper, less time-consuming and demonstrated higher performances than classical qPCR to estimate oseltamivir-resistant IV subpopulation percentages. This technique could be used to monitor the emergence of H275Y-NA mutation in immunocompromised patients. An early detection of oseltamivir-resistant population may improve the therapeutic management and decrease the rate of therapeutic failure.

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A new combination of old techniques for HIV disease progression – qRT-PCR and ONEp-PCR

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Background: Human immunodeficiency virus type 2 (HIV-2) have unique properties as a human pathogenic agent: is less efficient on developing pathologic manifestations, the infection is generally defined as less virulent and HIV-2 infected individuals have a lower viral burden, accomplishing to a lower transmission rate.

Primary and chimeric HIV-2 viruses have been shown to use alternative coreceptors beyond the main CCR5 or CXCR4 to enter host cells. These features seem to be related to a flexible envelope oligomeric structure. HIV coreceptor usage, cDNA integration, and HIV pathogenesis are mechanisms with poorly understood dynamics.

Sensitive methods are needed for quantifying gene expression and copy number in HIV infected cells. Combining qRT-PCR with ONE*p*-PCR (one primer-PCR), which is a relatively simple customized technique that can be used to investigate fingerprinting, polymorphisms, genomic instability in HIV infected cells, and has the potential to reveal associated markers, is a renewable resource for numerous studies in various fields of modern biology and medicine.

Findings: In this work it is shown that primary HIV-2 R5 and ROD/*env*R5 or ROD/*env*-R5/-X4 chimeric viruses have differential behaviour related to copy number integration and expression.

Conclusions: HIV-2 *env*-V1V2 region is responsible to trigger different signal pathways leading to *ccr5* and *env* expression, and *env* copy number, in infected human T-lymphocytes genomic DNA. These results also point out the potential usefulness of combining qRT-PCR and ONE*p*-PCR to detect changes in HIV proviral DNA within the infected cell's genome, as a new tool for HIV disease progression.

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Abstract no: 328
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Performance of the IDS-iSYS "walk away" immunoassay system for the determination of Epstein–Barr Virus (EBV) serological status

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Background: The combination of EBV specific serological markers is the best strategy to assess the immune status against EBV infection. Usually the simultaneous detection of IgM and IgG antibodies against the viral capsid antigen (VCA) and against the EBV nuclear 1Ag (EBNA1) is sufficient to differentiate between: absence of infection (AI), past infection (PastI) and primary infection (PrI). Here we report the performance of a new commercial automated immunoassay system, IDS-iSYS, for the detection of VCA IgM, VCA IgG and EBNA IgG on a large panel of samples.

Material and methods: A panel of 435 sera (426 patients) was retrospectively selected from our university hospital routine screening EBV serology.

The immunoassay routinely used in our laboratory for the detection of IgG and IgM antibodies against EBV (Enzygnost® Immunoassay, Siemens Healthcare Diagnostics;) and IgG antibodies against EBNA (BioMedicalDiagnostics) were used as "reference tests" to classify the 435 samples as: (i) seronegative (EBV IgG/IgM and EBNA IgG negative $n = 90$); (ii) past infection (EBV IgM neg, EBV IgG pos, EBNA IgG pos, $n = 108$); primary infection (EBV IgM pos and EBV IgG pos or neg, EBNA IgG neg n: 117).

Additionally, 22 sera (22 patients) with an "isolated EBV IgG" status and 15 sera (7 patients) with all the three markers detected as positive (EBV IgM/IgG pos and EBNA IgG pos) were re-classified as primary infection or past infection with further analysis (indirect immunofluorescence assay, heterophile antibodies detection) and tested with the IDS-iSYS system. Eighty-three serologically proven primary infections caused by other viruses (cytomegalovirus, hepatitis viruses, parvovirus. . .) were also used to test the IDS-iSYS IgM VCA cross reactivity.

Sensitivity (Sens.) and specificity (Spe.) of the three IDS-iSYS parameters were compared to the reference tests. The agreement between IDS iSYS EBV status obtained with the three iSYS markers and the expected EBV status obtained with the referent test was established in accordance with the criteria of interpretation of the manufacturers.

Results: IDS iSYs VCA IgM: Sens. during PrI = 97.5% (95%CI:92.6–99.4). Spe. during AI and PastI = 97,4% (95%CI:92.6–99). Spec with interfering sera from other viral primary infections =88.1% (95%CI:79.2–93.5).

IDS iSYS VCA IgG: Sens. during PrI and PastI = 71.2% (95%CI:62.3–78.7) and 98.2 (95%IC 95.4–99.3) respectively. Spe. during AI = 94.4% (95% CI:92.2–97.6)

IDS iSYS EBNA IgG: Sens. during PastI = 97.3 (95%CI 93.6–98.4). Spe. during AI and PrI = 98.9 (95%CI:93.9–99.8) and 90.1% (95%CI: 83.4–94.4) respectively.

Agreement for the determination of EBV status between IDS iSYS system and reference test = 84. 4 (95%CI:80.6–87.6).

Conclusion: The IDS- iSYS is a real "Walk-Away System", easy to use, fast and secure and appears to offer new reliable commercial immunoassays for the determination of EBV serological status.

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Abstract no: 335
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Evaluation of process control "Mengo Virus" using 3 RNA extraction kits and 2 different types of methods of one-step real-time RT-PCR in Donax sp (Palabritas)

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There are many problems to extract viral genetic material that is contaminating bivalve molluscs, this is due to bivalve molluscs, specifically the hepatopancreas, have many inhibitors to PCR, for that reason extraction methods should consider a virus process control used to measure the efficiency of extraction. In the market there are many commercial kits for extracting the nucleic acid of the virus and to perform One-step real-time RT-PCR, but most are not tested on bivalve mollusks, for this reason, the aim to evaluate the efficiency of extraction process control (Mengo virus) using 3 different RNA extraction kits and 2 treatments of One-Step real time RT-PCR. They were used to study 30 samples of hepatopancreas of *Donax sp.* (Palabritas) to which was added 10 μl of Mengo virus at a concentration of 1.6×10^4 particles/μl, and processing of the sample according to the ISO/TS 15216-2:2013, then RNA was extracted of each sample with the kits: 1. BioMerieux NucliSENS® system (bioMerieux SA, France), 2. PureLink™ RNA Mini Kit (Ambion-Life Technologies™, USA) and 3. Hugh Pure RNA Tissue Kit (Roche SA, Germany). Once extracted RNA was performed one-step real-time RT-PCR using the following treatments: 1. the UltraSense One-step qRT-PCR (Invitrogen, USA) kit according to the ISO/TS 15216-2:2013 was used and 2. Kit Mengovirus@ceeramTools™ (CEERAM, France) according to the manufacturer's specifications was used. It turned out that the measuring efficiency of the extraction process control (Mengo virus) the best method of extraction was BioMerieux NucliSENS® system with an efficiency 10 times greater than the second; and with respect to matters related to Kits One-step real-time RT-PCR it can be concluded that treatment 1 kit UltraSense One-step qRT-PCR has an efficiency of 32% over the Mengovirus@ceeramTools™ Kit.

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Abstract no: 337
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Ion Torrent next generation sequencing for accurate genotyping and detection of resistance associated variants in HCV and HIV

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Background: Detection of resistance-associated mutations is well established in HIV ART (as DRMs) and is increasingly used in HCV patients selected for treatment (as RAVs) with direct acting antiviral agents (DAAs). Both for DAA treatment and conventional interferon-based therapy accurate determination of HCV genotypes (GTs) is essential. Sanger sequencing has recognized limitations in sensitivity and turn around time. NGS provides excellent accuracy, speed and sensitivity enabling detection of rare mutants, HCV subtypes as well as mixed infections.

Objectives: To develop improved detection of clinically relevant viral mutations using ion torrent based NGS in an automated workflow.

Materials and methods: We have used NGS in combination with workflow automation on a newly developed platform based on the emotion 5075 system (Eppendorf, Germany) consisting of a continuous robotic process starting with sample extraction and RT-PCR followed by automated library preparation, Ion Torrent deep sequencing and direct online data analysis to determine HCV genotypes and RAVs as well as DRMs in HIV. We have employed target sequences from the HCV NS3, NS5A and NS5B regions. For HIV sequences in reverse transcriptase, protease and integrase were selected for NGS.

Results: We are reporting results from an evaluation study conducted on >200 HCV sera comparing HCV genotyping with line probe assay. Two cases of mixed GT infections were detected. Confirmation of discrepant results between NGS and line probing by Sanger sequencing indicated 100% accurate GTs by NGS whereas in several cases line probe results would have led to selection of suboptimal therapy regimens. In an HIV pilot study ($n = 112$ patients), comparing NGS results to TruGene sequencing the *Sentosa* SQ HIV Genotyping Assay detected 100% (199/199) of all mutations in the protease gene and more that 98% mutations (427/435) in the reverse transcriptase gene.

Conclusions: Given the crucial role of accurate sequencing analysis in HCV and HIV treatment management, workflow automated NGS appears as a highly reliable tool for differentiating HCV GTs and RAVs, which can help to prevent diagnostic errors potentially leading to suboptimal treatment.

Considering the pivotal role of DRMs in HIV patients under HAART the *Sentosa* SQ HIV Genotyping workflow appears as a valuable new tool for detecting clinically relevant HIV variants. Given its high sensitivity compared to Sanger based systems and the

comparatively short turnaround time of two days the workflow offers relevant improvements in HIV DRM detection.

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Abstract no: 351
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Mass spectrometry of influenza virus using clinically available MALDI-TOF platform

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Viral respiratory disease caused by influenza viruses has an important medical, epidemiological and economic impact on global population. Ideal screening assays for influenza viruses directly from clinical specimens must be not only of high sensitivity and specify, but also must have short turn-around times (less than two hours). In many laboratories, screening for influenza virus directly from clinical specimens is based on express Direct Fluorescent Antibody Assay, which is quite specific, but not very sensitive, when compared to RT-PCR (reverse transcriptase PCR). On the other hand, nucleic acid amplification assays, such as RT-PCR, are not characterized by quick turn-around times. Little is known if mass spectrometry technology may be used as an alternative screening approach for influenza virus identification directly from clinical samples.

The main objective of our study was to analyse mass spectra for influenza virus identification using a mass spectrometer, which is routinely used in our Clinical Microbiology Diagnostic Laboratory for bacterial or fungal identification. In this preliminary study, we used cell culture or egg-amplified influenza viruses as well as their corresponding recombinant neuraminidase (NA) and hemagglutinin (HA) proteins. Mass spectra were generated using clinically available Biomerieux Vitek® MS MALDI-TOF mass spectrometer. All proteins and whole viruses were pre-treated by lysis solution, sonication, boiling and microwaving. Influenza viruses and their corresponding HA and NA proteins were either directly applied on MALDI-TOF target or a minimal pre-treatment with either formic acid extraction and/or short trypsin digestion was used prior to target application of samples. Overall, the turn-around time for specimens was from less than an hour (without trypsin digestion) to three hours (with trypsin digestion). Mass spectra for H1N1, H3N2 and B influenza viruses as well as their corresponding HA and NA were analysed. We used VENN diagrams to manually analyze spectra of HA and NA and the corresponding whole virus in order to identify potential peak (m/z) candidates for the influenza virus identification by MS MALDI-TOF. On average, three to five peak candidates were identified for each influenza virus based on mass spectra analysis of HA, NA and entire influenza virus mass spectrometry.

Engineering and implementation costs for a mass spectrometer, which may be used in a Clinical Microbiology setting and yet possess a resolution capacity comparable to that of research use only platforms, is quite lengthy and financially demanding procedure. In this preliminary study we investigated whether already clinically available mass spectrometer may be used for influenza virus identification. We obtained a number of peak candidates, which may be used for a peak database creation in the future experiments. In the future, we are planning to identify influenza viruses directly from virus-spiked clinical specimens as well as from clinical specimens derived from symptomatic patients.

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Abstract no: 41
Presentation at ESCV 2016: Poster 49

Increasing blood safety by diagnosing Zika, Chikungunya and Dengue in times of massive outbreaks

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The Aedes trio (Dengue, Chikungunya and Zika) are arthropodborne viruses that are transmitted by mosquitos of different Aedes species (*Aedes aegypti, Aedes albopictus*). They have been reported in Africa, the Americas, Asia and the Pacific Islands. Dengue virus is a flavivirus, closely related to Zika whereas Chikungunya belongs to the alphaviruses. Dengue shares some clinical signs with Chikungunya and Zika and they can be misdiagnosed in areas where these arboviruses are common. As Dengue infection may cause a rash that could be confused with other diseases such as Chikungunya, Zika and measles, these other diseases do need to be ruled out. Diagnosis of Zika will first and foremost be by exclusion of other diseases such as Chikungunya and Dengue, based on symptoms and travel history. It is known that these diseases can also be transmitted by blood transfusion. Since a great proportion of infected persons are asymptomatic special care has to be taken in respect to blood safety.

Surveillance and testing algorithm for these three co-circulating arboviruses are needed since they show high impact on the socio economic burden in endemic countries. WHO proposed very recently a testing guidance for laboratory detection and diagnosis of these diseases. On 1 February 2016 the WHO declared a Public Health Emergency of International Concern (PHEIC) regarding a recent cluster of microcephaly cases and other neurological disorders and the possible association of these illnesses with Zika virus infections. The WHO recommended efforts towards improved surveillance of Zika virus which is only possible with an accurate diagnostic system for Dengue and Chikungunya as well.

Here we show how the BEP® III and BEP 2000 Advance® systems of Siemens Healthcare GmbH in combination with the Novagnost® ELISA assays can help in the management of outbreaks, proper diagnosis of individuals and surveillance of populations at risk.

The combination of highly sensitive and specific ELISA assays (Dengue IgG sensitivity >95%/specificity >95%; Dengue IgM sensitivity 82,3%/specificity >95%; Chikungunya IgG sensitivity >98.6%/specificity 100%; Chikungunya IgM sensitivity >98.8%/specificity 100%/Zika IgM sensitivity >100%/specificity 98.2%) fulfills the criteria of the WHO testing guidance for high throughput screening of these diseases and therefore seems to be an excellent tool for surveillance of blood products.

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Abstract no: 49
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Newly diagnosed HIV-1 cases: Decreased proportion of recent infections based on a multi-assay algorithm combining two serological assays

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Background: HIV surveillance requires monitoring of new HIV diagnoses and differentiation of incident and older infections. Accurate incidence estimates are needed to identify populations at increased risk of HIV acquisition, monitor the HIV/AIDS epidemic and evaluate interventions for HIV prevention. Unfortunately, serologic assays developed for cross-sectional incidence estimation often overestimate HIV incidence because some long term infections are classified as assay positive (incident). The use of multi-assay algorithms to estimate the incidence is a promising alternative approach.

Objectives: To develop a multi-assay algorithm which combines use of routine serological laboratory diagnostic tools for determination of accurate HIV-1 recent infection.

Study design: Newly confirmed HIV infections from January–April 2016 ($n = 163$) were extracted from the NVRL HIV Database. For each new HIV diagnosis, HIV INNO-LIA assay (LIA) results and risk factor data, where available, were obtained from the NVRL laboratory information system. Recent HIV infections were identified using the following criteria: Evidence of an HIV negative test in the previous 12 months, detection of p24 antigen on first diagnosis and application of the LIA HIV confirmatory assay banding pattern. All samples were also tested using the Sedia HIV Limiting Antigen Avidity assay (LAg). The assays were performed in both screening (tested in singlet) and confirmatory (tested in triplicate) modes. The normalised cut-off in the screening assay was ODn = 2 and in the confirmatory assay was ODn = 1.5. In order to develop a multi-assay algorithm (MAA) for determination of accurate HIV-1 recent infection, results of the LAg assay were combined with the LIA using Algorithm 15.1 developed by Schupbach J et al., 2015. These algorithms are derived from antibody reaction scores to the seven HIV antigen bands present on the LIA strip (sgp120, gp41, p31, p24, p17, sgp105 and gp36).

Results: All patients were HIV-1 subtype B. Excluding four patients who were receiving antiretroviral treatment, a subset of 31 patients had low avidity suggestive of recent infections according to the LAg assay (19.5%). Applying Algorithm 15.1, divided this group into 15 patients with recent infection (9.4%) (Group 1) and 16 with long-term infection (Group 2). Mann-Whitney test analysis showed that Group 1 patients had significantly lower LAg avidity (ODn: 0.370 ± 0.10) when compared with Group 2 patients (ODn: 0.672 ± 0.089, $p < 0.01$). HIV viral loads were measured in a subset of patients and tended to be higher in Group 1. Although there was no significant difference in age between the groups, 9 patients (56.3%) in Group 2 were older than all the patients in Group 1. Both groups comprised mainly males, 86.7% and 87.5% respectively and the predominant cohort was MSM, comprising 80% and 70% respectively.

Conclusions: In this preliminary study, the 2-assay MAA which combined LAg and LIA Algorithm 15.1 decreased the proportion of recent HIV infections from 19.5% to 9.4%. Identification of an MAA that can be performed entirely on stored serum or plasma samples and does not require CD4 cell count or HIV viral load has major implications for HIV surveillance. Nevertheless, it is intended now to combine the results of the MAA with clinical epidemiological data to provide the most accurate information on incident cases defined by risk groups.

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Lateral flow immunochromatographic assay for detection of Porcine Respiratory Reproductive Syndrome Virus type 1-specific antibodies

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded positive-sense RNA virus that belongs to the family *Arteriviridae*, order *Nidovirales* (Cavanagh, 1997). It was first discovered in 1987 in the USA (Hill, 1990) and was discovered later in Europe in 1991 (Collins et al., 1992). The pathogenic organism is one of the most economically noteworthy infectious diseases of swine in many regions of world (Christianson et al., 1992, Meulenberg et al., 1993). It is highly required to discriminate the genotype of PRRSV so that the appropriate control measure will be applied to the PRRSV infected farm to minimize economic loss. Therefore, we have developed the lateral flow immunochromatographic assay (LFIC) as a highly useful point-of-care testing for the early detection of antibodies induced only by PRRSV type 1.

Materials and methods: The type 1 PRRSV (Lelystad) recombinant nucleocapsid proteins were produced by using E. coli expression system. And used for immunization to the Balb/c mouse for generation of specific monoclonal antibodies against it. The recombinant nucleocapsid proteins were coupled to the gold nanoparticles (about 40 nm) in appropriate conditions as a detector, and the monoclonal antibodies were immobilized to the nitrocellulose membrane as a capture.

Results: This analysis of samples from PRRSV-negative field samples was used. A total of 126 pigs serum samples were tested by ELISA (IDEXX), IFA, and LFIC assay for detection of PRRSV type 1. The specificity was determined to be 124/126%. To evaluate the distinction between detection of PRRSV type 1 and type 2 antibodies, sera from 6 pigs infected with PRRSV type 1 (E38) and type 2 (LMY, PL97-1) were collected during days post infection (to 52 pi). In the case of the PRRSV type 1 antibodies, the reaction signals were detected at the test zone of this kit but no reaction signals were detected in PRRSV type 2 antibodies. In addition, to determine the assay at early stages of the infection, sera from 8 pigs infected with type 1 (LV) and type 2 (VR2332) were collected during days 9 pi. While standard methods showed low sensitivity rates before day 9 pi, LFIC assay detected seropositive samples at days 7 pi showing greater sensitivity at early stages of the PRRSV type 1.

Conclusions: In conclusion, the high consent between LFIC assay and commercial ELISA assay suggests that the LFIC assay is a very useful method for the early detection of pigs infected with type 1 PRRSV. Thus, the LFIC assay may be a useful alternative to the current diagnostic tools used to detect PRRSV type 1-specific antibodies [1,2].

Reference

[1] E. Brown, S. Lawson, C. Welbon, J. Gnanandarajah, J. Li, M.P. Murtaugh, E.A. Nelson, R.M. Molina, J.J. Zimmerman, R.R. Rowland, Y. Fang, Antibody response to porcine reproductive and respiratory syndrome virus (PRRSV) nonstructural proteins and implications for diagnostic detection and differentiation of PRRSV types I and II, Clin. Vaccine Immunol. 16 (2009) 628–635.

[2] J.Q. Chu, X.M. Hu, M.C. Kim, C.S. Park, M.H. Jun, Development and validation of a recombinant nucleocapsid protein-based ELISA for detection of the antibody to porcine reproductive and respiratory syndrome virus, J. Microbiol. 47 (2009) 582–588.

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Random access molecular diagnostics – Increased efficiency in laboratory workflow and translation of reduced result turnaround time to patient benefit

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The advent of molecular diagnostics for infectious diseases was accompanied by the ability to drastically reduce the time taken to produce a diagnostic result, with tangible benefits to the patient pathway such as rapid administration of treatment and timely infection control intervention. However, the majority of molecular diagnostic platforms for the diagnosis of infectious disease are reliant upon batch processing in order to maximise cost effectiveness or due to limited availability of staff. This batch processing workflow limits the possible reductions in result turnaround time and thus potential benefits to the patient pathway.

The DxN VERIS System by Beckman Coulter is a random access, real-time PCR analyser for the combined extraction and quantitative analysis of pathogen nucleic acid in clinical samples. The system is designed to reduce staff hands on time and enable real-time testing of clinical samples. In order to evaluate potential reductions in turnaround time to result, improvements in laboratory workflow and the opportunity to re-optimise the skill mix of laboratory staff the DxN VERIS System was evaluated in parallel with current testing methodologies at the Nottingham University Hospitals Microbiology department. Of the four assays selected for comparison (HIV, hepatitis B, hepatitis C and CMV quantitation), significant reductions were demonstrated across all. Average turnaround time reductions ranged from 36 h for CMV results, to 190 h for hepatitis B results and in most cases with VERIS analysis a result would have been made available to the requesting clinician on the same day as the sample request.

The issue of how result turnaround time directly affects the patient is often not considered when evaluating a diagnostic platform, but is becoming more important with recent initiatives to implement timely and appropriate antimicrobial treatment and necessary infection control precautions. The evaluation at Nottingham University Hospitals investigated this aspect further, evaluating individual patient cases and demonstrating how a reduction in turnaround time via random access testing could directly translate to a positive impact on the patient care pathway. Random access testing facilitates increased efficiency in the patient's care, timely intervention with appropriate antiviral therapy and prompt decisions for future patient management.

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Real-time multiplex PCR system detection of viruses and bacteria in blood from febrile infants <90 days of age

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Background: Fever in infants (<90 days old) is a common medical problem that accounts for a large proportion of pediatric emergency department visits. Because the symptoms displayed are often non-specific, it is difficult to distinguish between viral syndromes, serious bacterial infection (SBI), or non-infectious causes of fever. Clinicians currently rely on a combination of patient history, physical examination, and laboratory findings to identify patients that are at a high or low risk of SBI. While this methodology is intended to reduce unnecessary testing/treatment, most febrile infants still receive extensive evaluation as well as hospital admission until a bacterial infection can be ruled out. A rapid, easy-to-use, comprehensive diagnostic test could benefit patient care in this vulnerable population by potentially reducing antibiotic use or influencing hospital admission decisions.

To aid in the etiological identification for this non-specific syndrome, BioFire Diagnostics is developing the FilmArray® Febrile Infant (FI) Panel for use on the FilmArray System. The FilmArray FI Panel simultaneously tests for six bacteria and ten viruses using 200 μL of blood. Two minutes of hands-on time are required and comprehensive results are returned in about an hour.

Materials and methods: 245 blood specimens from infants, ≤90 days, with either a fever (reported temperature > 38.0 °C) or a blood/CSF culture where an SBI was suspected, were evaluated in this study. This study was approved by each participating sites institutional review board (IRB). Residual standard of care (SOC) specimens as well as prospectively collected samples were tested. Each blood sample was divided into two 250 μL aliquots for FilmArray FI testing and independent comparator PCR testing.

FilmArray FI results were compared to SOC laboratory results as well as verified with independent PCR comparator assays. Any discrepancies between the FilmArray FI Panel and comparator PCR were investigated with additional bi-directional PCR sequencing.

Results: There were 80 detections of virus or bacteria, across the 245 blood specimens (33% positivity). Seventy-nine percent (79%, 63/80) were viruses and 21% were bacteria (17/80). The most frequently detected virus was enterovirus (17), followed by human herpesvirus 6 (16) and adenovirus (7). The most frequently detected bacteria were *Streptococcus agalactiae* (6), *Escherichia coli* (4), and *Staphylococcus aureus* (3). Blood culture identified 17 bacteria, seven of which were common skin contaminants such as Coagulase-negative *Staphylococcus*. FilmArray FI and blood culture were in agreement of 4 of 8 shared results. Dual viral and bacterial infections were observed in eight samples. The FilmArray FI Panel

provided a positive microorganism identification in 21% (23/106) of cases where SOC test results were negative.

Conclusion: Testing of febrile infants with the FilmArray FI Panel resulted in a pathogen detection in 33% (80/245) of tests, most of which would not require antibiotic intervention. Additionally, the FilmArray FI Panel detected 17 bacteria directly from blood with no upfront enrichment step. These results suggest that the FilmArray FI Panel could be a useful system to rapidly aid in identifying pathogens causing fever in infants.

This abstract contains information regarding assays that have not been reviewed by regulatory agencies for *in vitro* diagnostic use.

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HIV incidence assays: Evaluation of three HIV Avidity enzyme immunoassays

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Background: The development of assays for detection of recent HIV infections is crucial for analysing trends in infection in different populations for surveillance and prevention measures. Several HIV Avidity assays have been developed to distinguish between recent HIV infections and long term established infections.

Objectives: To identify and validate a suitable method for determining recent HIV infections in Ireland and to incorporate this assay as a routine diagnostic tool in the NVRL.

Study design: We compared three currently available manual HIV avidity enzyme immunoassays: Sedia HIV Limiting Antigen Avidity assay (LAg), Sedia BED HIV-1 Incidence immunoassay (BED) and a modified Bio-Rad Genetic Systems HIV-1/HIV-2 plus O enzyme immunoassay. A total of 50 samples from the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) which included 15 recent and 35 long term HIV-1 infections were tested. All assays were performed in both screening (tested in singlet) and confirmatory (tested in triplicate) modes. The normalised cut-off in the screening assay was OD = 2 and in the confirmatory assay was OD = 1.5.

Results: All patients were HIV subtype B. The age range was 23-64 years and included 47 males. HIV viral loads ranged from 709 to 9.44×10^6 copies/ml. None of the patients were on antiretroviral therapy at the time of sampling. In the screening mode, the positive predictive value for the LAg, BED and Bio-Rad assays were 98%, 90% and 93.3% respectively, sensitivity was 100% for all assays however, specificity was 100%, 100% and 97.2% respectively. The modified Bio-Rad assay incorrectly identified 1 sample as recent infection. HIV viral loads were significantly higher in recent infections ($p < 0.001$) and seroconversion intervals were longer in long term infected individuals ($p < 0.001$). Chi-square analysis revealed that more long term infected patients had Fiebig stage of V+ compared to recently infected individuals ($p < 0.001$). Significant correlation was observed when 24 samples from Irish patients were tested at the NVRL and Public Health England laboratory, $r^2 = 0.96$, $p < 0.001$.

Conclusions: The method identified for use in the NVRL is the Sedia HIV-1 Limiting Antigen Avidity enzyme immunoassay. This method is recommended by the WHO and is used by Public Health England to distinguish recent HIV infection from long-term infection. This assay will be used in the NVRL to test all new HIV diagnoses from January 2016 onwards.

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Evaluation of the H-DiaCMVQ kit® for detecting and quantifying CMV-DNA in plasma and in whole blood samples

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Aims: To validate the analytical characteristics of the H-DiaCMVQ kit® for the detection and the quantification of CMV-DNA in plasma and in whole blood. To assess the clinical performances and to analyze the influence of blood compartment by comparing the H-DiaCMVQ kit® and the in-house method on 150 samples.

Material and methods: Whole blood samples were tested with the in-house method. 50 positive (mean viral load: 2.79 $\log_{10}$ IU/ml, range: 6–1.85 $\log_{10}$ IU/ml) and 100 negative samples were selected for analysis and plasma collection.

Nucleic acids were extracted with the MagNA Pure 96 DNA and Viral NA Small Volume kit® on the MagNA Pure 96™ instrument according to the Pathogen Universal 200 protocol (plasma) and DNA Blood SV protocol (whole blood) (Roche Molecular Diagnostics, Meylan, France).

For comparison, samples were tested with the reference method targeting the UL83 (limit of detection 74 IU/ml (1.87 $\log_{10}$ IU/ml) and with the H-DiaCMVQ kit® (Diagenode, Seraing, Belgium), both on the Light Cycler 480™. Results were calculated in $\log_{10}$ IU/ml.

Results: The analytical performances of the H-DiaCMVQ kit® were very satisfactory on either plasma or whole blood: specificity was 100% and a very high range of linearity was obtained.

Intra-assay reproducibility was 0.20 and 0.03 in two plasma samples (3.04 and 6.10 $\log_{10}$ IU/ml) it was 0.33 and 0.07 in two whole blood samples (3.17 and 6.87 $\log_{10}$ IU/ml). Inter-assay reproducibility was 0.30 and 0.13 in two plasma samples (3.58 and 5.38 $\log_{10}$ IU/ml) it was 0.47, 0.19 in two whole blood samples (3.62 and 5.51 $\log_{10}$ IU/ml).

CMV-DNA correlated well in plasma between both methods ($n = 24$): $r = 0.82$, $p < 0.0001$, (slope of Deming regression 0.912 [CI 95%: 0.63–1.19] and y-intercept 1.12 [CI 95%: 0.30–1.94]. Similarly CMV-DNA correlated well in whole blood ($n = 36$): $r = 0.86$, $p < 0.0001$, (slope of Deming regression 1.071 [CI 95%: 0.86–1.28] and y-intercept 0.84 [CI 95%: 0.18–1.5].

We analyzed the influence of the blood compartment by comparing the results obtained on whole blood with the reference method with those obtained with the H-DiaCMVQ kit® on corresponding plasma samples ($n = 150$). 142 samples gave concordant results (36 positive and 106 negative). Eight samples gave discrepant results: 2 samples were positive in whole blood and

negative in plasma (3.54 and 3.83 $\log_{10}$ IU/ml) and 6 gave the opposite results (mean viral load: 2.73 $\log_{10}$ IU/ml). All positive ($n = 36$) samples were analyzed. Linear regression analysis showed a good correlation between the two methods: $r = 0.75$, $p < 0.0001$, (slope of Deming regression 1.299 [CI 95%: 0.900–1.698] and y-intercept 0.49 [CI 95%: −1.908 to 0.9199]. The Bland-Altman representation showed that the CMV-DNA quantitation in whole blood gave higher virus loads than did the CMV-DNA quantitation in plasma: the average deviation was −0.54 $\log_{10}$ IU/ml (SD = 0.60).

The influence of the blood compartment was also analyzed by comparing the virus load kinetics for successive samples selected from four immunosuppressed patients (16 samples). Overall results showed similar patterns with variation in the same direction. Whole blood was the only compartment that tested positive in one patient for very low virus loads (2.02–2.78 log $\log_{10}$ IU/ml).

Conclusion: The H-DIACMVQ kit® provides precise, reproducible results and it satisfies quality requirements for routine monitoring of DNA-CMV in plasma or whole blood samples.

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Use of recombinant virus technology to produce non-infectious, whole process controls for emerging viruses such as Ebola, Chikungunya, Dengue-2, Norovirus GII, MERS-CoV and Zika

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Background: Outbreaks of viral communicable disease and appearance of new viral strains can represent public health emergencies. As diagnostic laboratories and test developers design, manufacture and validate diagnostic assays to prepare for these threats, positive reference materials are needed. SeraCare has developed AccuPlex™ recombinant virus technology to produce whole process reference materials that mimic clinical samples. They are mammalian virus products and are non-infectious. AccuPlex technology was used to develop quality controls for amplified nucleic acid tests for the emerging viruses Ebola, Chikungunya, Dengue-2, Norovirus GII, MERS-CoV and Zika as well as drug resistant HIV-1. Here we demonstrate the performance of these quality control materials and show that they have an extended stability at 2–8 °C and do not require freezer storage.

Methods: AccuPlex™ controls for RNA viruses employ engineered Sindbis virus, and a portion of the Sindbis structural genes are replaced with up to ~4000 bp of the diagnostic targets of interest. Where the diagnostic targets are well defined, those regions were incorporated into one recombinant virus. For example, the recombinant Chikungunya reference material contains portions of the NSP1, NSP2, NSP4, Capsid, E3 and E1 genes, and is based on the sequence of strain IND-06-Guj. The recombinant Dengue reference material contains portions of 3′ UTR, NSP5, Capsid, and E1 genes from serotype 2. Recombinant Norovirus, Ebola, and MERS-coV reference materials follow a similar design scheme.

However, when diagnostic targets are undefined, as is the case for Zika, a different design scheme is required. The entire Zika genome was divided into four segments and each segment was used to generate an AccuPlex recombinant virus. The Zika reference material therefore is a mixture of four distinct AccuPlex recombinant viruses. Dividing the pathogen's genome among multiple constructs ensures each recombinant virus is not functional. Additional safety features such as gene truncation, multiple stop codons and frame shifts are also used and the products are heat treated for viral inactivation.

Results: Recombinant AccuPlex viruses were diluted in defibrinated plasma or other commutable matrices and characterized by Digital PCR using pathogen specific primers and probes. The target concentration range of the reference materials is from 5E + 05 copies/mL for recombinant Ebola, to 5E + 06 copies/mL for many of the other viruses. Functional testing of the reference materials on Altona RealStar RT-PCR Kits as well as Primer Design Ltd GeneSig Advanced kits showed positive detection. The recombinant viruses gave cycle threshold values (Ct) on these assays consistent with a low positive control (Ct of 27–31.5). Accelerated stability studies indicate that the product is stable at 4 °C for at least two years. Real time stability data at Room Temperature has been collected through 20 months and updated stability data will be presented.

Conclusions: SeraCare has developed stable, well-characterized whole process controls for pathogenic viruses. These reference materials will enable laboratories to validate tests and train technicians to ensure preparedness for outbreaks. These products demonstrate the utility of recombinant virus technology to produce non-infectious controls for select agents and viruses difficult to source or propagate.

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Abstract no: 67
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Characterisation and standardisation of Qnostic products in the absence of higher order standards

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Introduction: Viral load determination plays a critical role in clinical diagnostics and a central role in monitoring patients' response to treatment and disease progression. However, true transferability of results remains elusive due to the lack of inter-laboratory standardisation. Where available, International Standards have helped to facilitate data comparison but where there is no standard or Certified Reference Material available assay variation obscures meaningful comparison of results at the technology and laboratory level. The use of characterised control materials with known performance characteristics would allow for objective comparisons between laboratories and assays used. In this study we evaluated the use of digital PCR (dPCR) to quantify control materials for four viral targets (Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), JC Virus (JCV) and BK Virus (BKV)), and established performance across the top five available commercial assays in clinical use for each. International Standards are available for CMV and EBV but not for JCV and BKV. Digital PCR permits the characterisation of control materials without the requirement of a standard or certified reference material thereby allowing direct comparison of results between laboratories.

Methods: Control materials for each of the 4 viral targets (Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), JC Virus (JCV) and BK Virus (BKV)) were prepared at a single titre in human plasma at a concentration that fell within the linear range for most assays in use. The controls were characterised internally using both an in-house qPCR based method and digital PCR (BioRad QX200). Blind panels were provided to laboratories participating in the study in 2015. Laboratories were asked to treat the materials as they would a clinical sample and to return quantitative data along with information on the assay workflow used to generate the results.

Results: The data from 2015 was anonymised, collated, and outliers removed. The data were split into categories based on the assay manufacturer who provided the amplification reagents. Outliers were assessed through the application of Grubbs' analysis to each assay manufacturing group. Datasets were considered suitable for inclusion if more than one laboratory reported data using the same assay manufacturer after removal of non-compliant data and outliers. For CMV, 120 quantitative datasets across 5 assays were returned, 106 for EBV, 132 for JCV and 144 datasets for BKV. Assay manufacturing group datasets were compared against the group consensus and the dPCR assigned values.

Conclusions: The comparison of inter-laboratory data for viral load determination is limited in the absence of certified control materials or international standards due to potential assay variation. The aim of this study was to compare the quantitative performance of dPCR against the key commercial assays available for the four viral targets. The results showed that the dPCR results aligned closely with the quantitative values determined using commercial assays and the in-house qPCR assay independent of an International Standard being available. Historical assay data (not shown) support this data and show the close alignment between qPCR and dPCR assays. Digital PCR allows quantitation without the need for a calibrated standard and from the preliminary data presented here indicates a method by which to calibrate controls allowing comparison of results between laboratories.

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Comparison of two multiplexed PCR assays for respiratory virus detection in ICU patients: FilmArray® respiratory panel and Allplex™ respiratory full panel

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Background and objectives: The FilmArray® Respiratory Panel (BioFire Diagnostics, LLC, Salt Lake City, UT, USA, a bioMérieux Company) is a multiplexed automated PCR assay that integrates specimen processing, nucleic acid amplification, and detection into a pouch, and detects 17 respiratory viruses plus three bacteria. We compared it to another multiplexed PCR assay, Allplex™ Respiratory Full Panel (Seegene, Inc., Seoul, Korea) composed of 4 different panels in a multiplex One-step Real-time RT-PCR assay to detect and identify 16 respiratory viruses and 7 bacteria in patient's specimens. We conducted a study to evaluate the performance of the FilmArray® compared to the Allplex™ for the detection of respiratory viruses from different respiratory specimens in ICU patients. Bacteria were not taken into account in this study.

Methods: A prospective comparative study was carried out in 50 respiratory specimens (nasal swabs, nasopharyngeal lavage, bronchoalveolar lavage, bronchoaspirate and sputum) collected from ICU patients between March and May 2016. One aliquot was processed with the Allplex™ and a second aliquot was tested by the FilmArray® assay. For the Allplex™ assay, viral nucleic acid was extracted using the BioRobot EZ1® (Qiagen). Both assays detect influenza A (Flu A; seasonal H1, subtype 2009 H1 and H3) and influenza B (Flu B), respiratory syncytial virus (RSV), adenovirus (AdV), human rhinovirus/enterovirus (HRV/E), parainfluenza 1-4 (PIV 1-4), human metapneumovirus (HMPV) and coronaviruses (CoV) NL63/229E/OC43. Additionally, FilmArray® detects CoV HKU1 and Allplex™, bocavirus (BoV). For discrepant results, virus-specific RT-PCR was performed (RealStar® RT-PCR Kits, Altona Diagnostics, Hamburg, Germany).

Table 1
Discrepant results between FilmArray® Respiratory Panel and Allplex™ Respiratory Full Panel, and virus-specific RT-PCR results.

Sample	FilmArray® respiratory panel	Allplex™ respiratory full panel	Specific RT-PCR
Nasal swab	AdV	Negative	Negative
Nasal swab	Flu A 2009	Negative	Flu A 2009
Nasal swab	AdV	Negative	Negative
Nasal swab	Flu A 2009	Negative	Flu A 2009
Sputum	Negative	Flu B	Negative
Nasal swab	Negative	Flu B	Negative
Nasal swab	Negative	AdV	AdV
BAL	Negative	AdV	AdV

Results: Of the 50 specimens tested, both assays agreed on 21 negative and 21 positive respiratory specimens. Discrepant results (8) agreed with specific PCR in 4 for FilmArray® and in 4 for Allplex™ (Table 1). The FilmArray® showed a 92.6% sensitivity and 91.3% specificity compared to 92.3% sensitivity and 88.5% specificity for Allplex™.

Conclusions: The FilmArray® is a useful, easy-to-perform assay for detecting respiratory viruses in ICU patients. The Allplex™ requires additional RNA extraction time and more hands-on time, making it longer to perform. However, as the FilmArray® processes one single sample at a time, this increases the time of successive results when several samples arrive to the laboratory with a short difference in arrival time, but this point is solved by new platforms available.

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Evaluation of three different sample populations on a new multiplex BioPlex®2200 assay for the detection of measles, Mumps, and Varicella-Zoster virus IgM antibodies

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Background: Measles, mumps, and varicella are three of the most highly infectious diseases. Worldwide outbreaks continue in many countries despite aggressive vaccination campaigns. Early diagnosis of these diseases improves patient management and helps prevent outbreaks from spreading. To assist clinicians in making quick and accurate diagnoses, Bio-Rad Laboratories is developing a new assay used for the identification of IgM class antibodies to measles, mumps, and varicella-zoster virus (VZV) in human serum or plasma. The new BioPlex 2200 MMV IgM assay produces three discrete results from a single multiplexed test reaction and is being developed to accommodate a wide variety of sample types to facilitate diverse testing situations.

Methods: Retrospective samples positive by Diasorin Liaison assays (measles $n = 104$, mumps $n = 183$, and VZV $n = 64$), a test ordered sample population comprised of samples from a European reference laboratory (measles $n = 300$ mumps $n = 300$, and VZV = 300), and samples from a healthy population made up of sam-

ples from blood bank donors in the United States (measles $n = 399$, mumps $n = 399$, and VZV $n = 399$) were analyzed with the BioPlex 2200 MMV IgM assay. The results were compared to the DiaSorin Liaison measles IgM, mumps IgM and VZV IgM assays. The BioPlex MMV IgM assay was further evaluated for imprecision around the cutoff. Time to first result and throughput were also observed.

Results: Among the presumptive positive sample population, the BioPlex MMV IgM assay showed a positive agreement of 96.1%, 92.3%, and 95.2% for measles, mumps and VZV IgM, when compared to the Liaison Measles IgM, Mumps IgM and VZV IgM assays. The test ordered sample population had a positive prevalence of 1.0%, 5.0%, and 2.0% for measles, mumps, and VZV IgM respectively. Among the sample set taken from a healthy population the MMV IgM assay showed a negative agreement of 99.5%, 99.2%, and 100.0% for measles, mumps and VZV IgM respectively. The imprecision for positive samples was shown to be between 4.1 and 8.5% for measles IgM, 4.4–10.7% for mumps IgM, and 3.4–8.2% for VZV IgM. The standard deviation of results from the negative samples was <0.05 SD across all three analytes. The MMV IgM assay has a time to first result of 45 min, throughput of 63 samples per hour, and offers up to 189 results per hour by utilizing multiplex technology.

Conclusions: The data demonstrate the BioPlex 2200 MMV IgM assay is comparable to commercially available assays and that this new assay meets laboratory needs for precision and throughput. By combining the three analytes together in a single test reaction, the BioPlex 2200 MMV IgM assay provides a complete and more efficient method to assist in the diagnosis of these highly contagious diseases.

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Abstract no: 80
Presentation at ESCV 2016: Poster 60

Paper-based point-of-care testing for cost-effective diagnosis of acute dengue infections

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Dengue is a serious healthcare concern in tropical and subtropical countries. Although well-established laboratory tests can provide early diagnosis of acute dengue infections, access to these tests is limited in developing countries, presenting an urgent need to develop simple, rapid, and robust diagnostic tools. Paper-based point-of-care (POC) devices, are typically rapid, cost-effective and user-friendly, and they can be used as diagnostic tools for the prompt diagnosis of dengue at POC settings. The early and prompt diagnosis is crucial to improve patient management and reduce the risk of severe dengue complications.

Here we have developed and evaluated a wax-printed paper-based device for the detection of the non-structural NS1 dengue viral protein in blood and plasma. The requirements to obtain both the highest specific signal and the lowest background have been studied. The raw materials quality and the washing steps have been determined as crucial. With the developed method we were able to detect specifically in 5–6 min less than 10 ng/mL of protein in various sample types. The read of the results was simplified by using a dedicated application on a smartphone.

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Abstract no: 86
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Loop-mediated isothermal amplification for point-of-care diagnosis of viral respiratory tract infection in childhood

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Background: Acute respiratory tract infection (ARTI) caused by respiratory syncytial virus (RSV) is a frequent cause of hospitalization in young children. The prevention of nosocomial infection therefore is crucial to reduce morbidity and mortality in severely ill patients.

Objective: The aim of this study is to apply novel isothermal amplification methods for point-of-care testing in children hospitalized with ARTI.

Methods: We evaluated a novel DNA amplification technique, called "Loop-mediated Isothermal Amplification" (L-AMP) allowing highly sensitive detection of RSV from purified viral RNA within approximately 30 min at comparably low costs. To evaluate the test results we compared them with real-time PCR as a gold standard. Nasopharyngeal swabs (mSwab, Copan, Italy) and clinical data were obtained from hospitalized children who presented with symptoms of ARTI.

Results: The L-AMP test was first evaluated using patient samples in a laboratory setting during the winter season 2014/2015 and showed a test sensitivity of 73% compared to 58% sensitivity of the conventional RSV rapid antigen detection test. In winter season 2015/16, we transferred the technology to a routine application in a point-of-care setting at the Paediatric Department of the University Hospital Heidelberg. From November 2015 until April 2016 336 swabs were collected, of which L-AMP results were obtained for 326 samples. Mean age of all children was 21.9 months and 43.5% were female.

In total 108 swabs (32.1%) were RSV-positive. The L-AMP test showed a sensitivity of 70.2% and a specificity of 96.8%.

Conclusion: This innovative approach could substantially enhance the accuracy to detect ARTIs in a point-of-care setting.

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Abstract no: 91
Presentation at ESCV 2016: Poster 62

Comparison of the performances of the three nucleic acid extraction systems: The Roche Magna Pure LCTM System, the Biomerieux easyMAG® system, and the new Biomerieux eMAG™ System

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Quantification by qPCR of Epstein-Barr DNA load in whole blood (EBV-L) is required for the monitoring of post-transplant lymphoproliferative disorder risk or adjustment of the immunosuppressive regimen. It may also be useful in other EBV-associated diseases. EBV-L measurement is not currently standardized but automated system for DNA extraction and commercial assays could help to obtain more reliable results in the routine setting of non-specialized laboratories. Here we compared the performances of three automated extraction systems: NucliSENS easyMAG® and eMAG™ (bioMérieux) and MagNA Pure LC (Roche). eMAG is the new generation of easyMAG: eMAG provides full automation while keeping high flexibility in term of sample management.

86 samples were collected from the routine of the laboratory before any congelation and DNA was extracted using the three automated systems. EBV-L was measured on a LC480 (Roche applied science) by using the EBV R-gene quantification kit (bioMérieux).

Qualitative analysis showed that the overall concordance was 78% between the 3 extraction methods. 11 results of 86 (13%) were discordant between MagNA Pure (MP) and eMAG (8 positives with eMAG/negatives with MP and 3 positives with MP/negatives with eMAG); 22 results of 86 (25%) were discordant between MP and easyMAG (EZM) (11 positives with EZM/negatives with MP and 11 positives with MP/negatives with EZM); and 23 results of 86 (27%) were discordant between eMAG and EZM (9 positives with EZM/negatives with eMAG and 14 positives with eMAG/negatives with EZM. All discordant results were below 2000 copies/mL.

Blant–Altman analysis showed that few samples were quantitatively discordant (variability > [mean difference]/2 ± 1.96 standard deviation): 1 of 29 (3.4%) for eMAG/MP comparison; 1 of 27 (3.7%) for EZM/MP; and 1 of 35 (2.8%) for eMAG/EZM. Blant–Altman analysis also showed that EBV-L obtained after MP extraction were higher than after eMAG extraction (mean differences = 0.2 log copies/mL) or EZM extraction (mean differences = 0.29 log copies/mL). Nevertheless, the final sensitivity is not impacted and this would not change the therapeutic management of patients: 22% of EBV-L obtained after MP extraction were 0.5 log copies/mL higher than after eMAG or EZM extractions.

In conclusion, the new eMAG extraction system fulfills the extraction performances for whole blood samples. The 3 automated systems allowed a reliable extraction of EBV DNA in whole blood with similar qualitative and quantitative performances. Discrepancies were mostly observed for low viral load. This emphasizes the current difficulty to compare EBV-L measured with different technologies, even statistically well correlated and suggest that the monitoring of EBV-L should be manage with a single method.

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Abstract no: 94
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Comparison of the Hologic Aptima HCV Quant Dx assay to the Roche COBAS Ampliprep/COBAS TaqMan HCV Test v2.0 for the quantification of HCV-RNA in plasma samples

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Background: Monitoring of HCV RNA levels remain useful in evaluating antiviral treatment in chronic HCV infection. Variations in performance of different tests may impact clinical decisions and knowledge of the analytical performance of tests is therefore important for clinical care.

Objectives: To compare the analytical performance of the APTIMA HCV Quant Dx Assay (APTIMA) and the COBAS Ampliprep/COBAS TaqMan HCV Test v2.0 (CAPCTM) for the quantification of HCV RNA in plasma samples.

Study design: The performance of the two tests was compared on 125 archived clinical plasma samples of known genotypes, and on dilutions series in six replicates of clinical samples of genotype 1a, 2b, 3a and 4a.

Results: Mean bias in quantification between the two test (APTIMA–CAPCTM) was 0.13 Log IU/mL (SE: 0.32 Log IU/mL) and changed little when results were stratified on genotypes (1a (N = 35): 0.26 Log IU/mL (SE: 0.29); 1b (N = 27): 0.23 Log IU/mL (SE: 0.25); 2b (N = 12): 0.31 log IU/mL (SE: 0.30); 3a (N = 36): −0.12 Log IU/mL (SE: 0.27). Although the two tests were highly correlated (R = 0.977), Deming regression showed that APTIMA quantified higher than CAPCTM for high viral loads. In the dilutions series the APTIMA test was linear with slopes very close to the expected (1a: 1.01; 2b: 1.02; 3a: 1.02; 4a: 1.00). For all four genotypes tested CAPCTM yielded slopes less than one (1a: 0.96; 2b: 0.94; 3a: 0.87; 4a: 0.93). The APTIMA assay appeared at least as sensitive as the CAPCTM test detecting for all four genotypes more replicates than the CAPCTM test. Precision of both tests was comparable with %CV less than 5% for HCV RNA levels above 100 IU/mL.

Conclusion: The APTIMA assay and the CAPCTM test are highly correlated. Both tests are sensitive and precise. Linearity of the APTIMA test is excellent and the test will be useful to monitor therapy responses during antiviral treatment of chronic HCV infection.

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Abstract no: 95
Presentation at ESCV 2016: Poster 64

Comparison of the Hologic Aptima HIV-1 Quant Dx assay to the Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test v2.0 for the quantification of HIV-RNA in plasma samples

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Background: Monitoring of HIV-1 RNA levels is the most important parameter for assessing efficacy of antiviral treatment in individuals infected with HIV-1. Knowledge of the performance of different tests for the quantification of HIV-1 RNA is therefore important for clinical care.

Objectives: To compare the analytical performance of the APTIMA HIV-1 Quant Dx Assay (APTIMA) and the COBAS Ampliprep/COBAS TaqMan HIV-1 Test v2.0 (CAPCTM) for the quantification of HIV-1 RNA in plasma samples.

Study design: The performance of the two tests was compared on 216 clinical plasma samples, on dilutions series in seven replicates of three clinical samples of known subtype (A1, B, CRF01AE) and on ten replicates of the Acrometrix High (appr. Log 6 cp/mL) and Low Positive Control (appr. Log 2 cp/mL).

Results: Bland–Altman analysis of 130 samples with quantitative results in both tests did not show indications of gross mis-quantification of either test. A tendency of the APTIMA assay to quantify higher at high viral load compared to the CAPCTM was observed in Bland-Altman analysis, by Deming regression (Slope 1.10) and in dilution series where the difference was most pronounced for the subtype B sample. Precision evaluated using the Acrometrix Positive Controls was similar for the High Control (CV: 1.2% vs. 1.3%; APTIMA assay vs. CAPCTM test, respectively), but differed for the Low Positive Control (CV: 17.9% vs. 7.1%; APTIMA assay vs. CAPCTM test, respectively). However, this did not impact categorization of 146 clinical samples with low viral load at neither the 50 cp/mL nor 200 cp/mL level.

Conclusion: The APTIMA assay and the CAPCTM test are highly correlated and are useful for monitoring HIV-infected individuals.

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Abstract no: 98
Presentation at ESCV 2016: Poster 65

Ensuring the quality of polyomavirus diagnosis in immunocompromised patients

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Background: The reactivation of polyomaviruses poses a significant risk in h immunocompromised patients. In the case of kidney transplants, renal dysfunction and graft loss caused by BKV associated nephropathy (BKVAN) have been documented; similarly, the onset of progressive multifocal leukoencephalopathy, a demyelinating disease of the CNS has been shown to be caused by JCV reactivation in patients undergoing immunosuppressive therapies.

The clinical application of nucleic acid amplification technologies (NAT) for BK and JC viral load assessment is integral to patient management. Whilst the employment of NAT assays provides a rapid means of viral load quantification, it has been demonstrated for BK virus that the establishment of a universal patient treatment threshold for BKVAN diagnosis, cannot be reliably achieved without an effective means of standardising assays.

Results: As part of a project to establish a multiplex reagent suitable as an in-run control for 11 different viruses typically associated with immunosuppression, we undertook a collaborative study. Laboratories were asked to determine viral load measurements of JC and BK viruses, using a range of commercial and in-house assays. For JC virus, all laboratories submitted results in "copies/ml" that demonstrated very good intra-laboratory reproducibility (<1Ct = <0.3 log10 copies/ml). However, the inter-laboratory analyses revealed poor comparability of data between laboratories (> 9Ct = ~3 log10). For BK virus, intra-laboratory variability was greater, whilst overall inter-laboratory was similar to that for JC virus.

To further address the challenge of effective calibration of assays for JC and BK viruses, we worked with the WHO's Expert Committee for Biological Standardisation to develop materials and undertake international collaborative studies to establish the 1st WHO International Standards as primary order calibrants for JCV and BKV NAT assays. Candidate viral preparations of JC and BK were sent to laboratories for assessment. In these studies the reported data ranges were 3.50–9.08 and 3.60–8.33 $\log_{10}$ copies/ml/NAT detectable units for JC and BK respectively across the participating laboratories, concurring with the findings of the CVN collaborative study.

By employing the basic principles of biological standardisation and expressing the data as a relative potency in comparison to a common reference, we show a reduction in the variability of data amongst all participating laboratories to <1.5 Log_{10} copies/ml/NAT detectable units.

Conclusions: The use of higher order calibrants when establishing assays and the regular use of external in-run controls that are calibrated against the International Standard will improve the comparability of NAT assays for polyomaviruses in a manner that has been achieved for blood borne viruses.

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Abstract no: 127
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Microbiological evaluation of respiratory tract infections in pilgrims returning from countries affected by Middle East respiratory syndrome coronavirus (MERS-CoV)

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Since September 2012, the World Health Organization (WHO) has been notified of 1728 laboratory-confirmed cases of infection with Middle East respiratory syndrome coronavirus (MERS-CoV), including at least 624 related deaths (disease outbreak news of April 26, 2016). Although MERS-CoV appears to be transmitted through respiratory droplets between humans with close contact, dromedary camels are likely to be a zoonotic source of MERS-CoV infection in humans. Early detection of MERS-CoV infection among international travelers exposed to camels or healthcare facilities in the Middle East remains essential. All travelers returning from MERS-CoV-affected areas to Paris (France) are given particular attention and those with fever and/or respiratory symptoms are referred to a dedicated infectious disease unit as the Infectious Disease Department of La Pitié-Salpêtrière University Hospital in Paris. The aim of this study was to investigate the microbiological etiologies of respiratory tract infections (RTI) among these specific travellers from the beginning of the 2015 Hajj and Umrah pilgrimage period (September 2015) to April 2016.

Upon admission, patients were isolated and nasopharyngeal swabs, sputum samples and, for persons on ventilators, bronchoalveolar lavage specimens were collected by trained nurses. We examined which etiological respiratory pathogens were identified during screening for MERS-CoV in symptomatic travellers returning to Paris during September 2015 to April 2016 period, from MERS-CoV endemic regions (published WHO bulletins). Firstly, samples were screened with a specific MERS-CoV real-time reverse transcription PCR targeting region upstream of the E gene (upE), as recommended by WHO. The second step of the etiologic diagnosis entailed an investigation for other respiratory viruses (influenza A/B viruses, respiratory syncytial virus, metapneumovirus, rhinovirus-enterovirus, parainfluenza viruses, other human coronaviruses) using Respiratory MWS r-gene® kits (bioMérieux) and for bacteria using standardized culture procedures.

A total of 31 symptomatic travellers mainly returning from Saudi Arabia (mean age 63.1 years, range 21–92 years; 58% male) were included during the study period and overall 48 respiratory clinical specimens were collected. None of the tested specimens were positive for MERS-CoV. Since a negative result should not absolutely rule out the possibility of MERS-CoV infection, notably if specimen is collected late or very early in the illness, some patients were screened twice. The vast majority of viral RTI, sometimes associated with bacteria superinfection, in these pilgrims returning home, were due to seasonal influenza A viruses (29%), rhinoviruses (23%), and other coronaviruses (7%) distinct from the MERS-CoV. Four patients were presenting acute lobar pneumonia, none were formally diagnosed. However, all were cured with antibiotics, as the presentation suggested pneumococcal infection. One case of Q fever, another known zoonosis transmitted by dromedary camels, and one case of *Legionnella pneumophilia*-associated disease were diagnosed among tested pilgrims.

Continuous surveillance should be implemented to ensure the timely detection of possible imported cases of MERS-CoV and their immediate isolation in order to avoid secondary cases. However, clinicians should be aware that influenza viruses and rhinoviruses are the most commonly identified pathogens in returning pilgrims with acute RTI.

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Abstract no: 136
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Determination of genotype distribution and the various polymorphisms in cytomegalovirus (CMV) strains

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Keywords: Genotyping; CMV; Phylogenetic analysis

Human cytomegalovirus has different genotypes, by determining these genotypes in different disease groups, the association of one and more than one infections can be found. In genotyping, frequently seen genetic polymorphisms gB (UL55) and gH (UL75) performed in envelope's glycoprotein. In our study phylogenetic analysis of 50 CMV (+) patient's gB and gH gene regions were done. In this study DNA sequence analysis performed and the result was evaluated by MEGA 6.0 program. According to phylogenetic analysis the results were; 48–50 patient gene region were amplified, 23 (%48) of these patients were gB1, 8 (%16) were gB2, 11 (32%) were gB3 genotype and one patient was gB4. According to UL 75 (gH) gene region the patientsgenotype was observed as; 6 (%12) were gH1 and 44 (%88) were gH, while in five of patients gB2/3 mix genotype was found (Table 1). According to gene regions, gB1 and gH2 were reported in more ratios.

According to sequence analysis results more polymorphism was observed. In the polymorphisms, the peptide change which belong to gB region frequently seen in gp166 while gH region is observed in general. We found S51Stop, K56E and T57H polymorphisms in all of the our patient's gH region, which is not found in the previous studies. The reason behind not finding S51Stop, K56E and T57H polymorphisms in previous studying is these polymorphisms are

Table 1

		Genotype (*n*/%)				
Gene region	Patients' numbers (*n*)	1	2	3	4	2/3
UL55 (gB)	50	23 (%46)	8 (%16)	11 (%22)	1 (%2)	5 (%10)
UL75 (gH)	50	6 (%12)	44 (%88)	–	–	–

particular to the area and this reveal it on the clinical features which increases its important in the studying.

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Abstract no: 142
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Appropriate diagnosis of Zika virus infection: Italy North-West experience

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Background: After large outbreaks occurred in Micronesia, 2007, and in the Pacific Area, 2013, Zika virus (ZIKV) was reported in Brazil in early 2015 and subsequently in the Americas and Caribbean. Autochthonous cases of ZIKV infections have been worldwide reported from at least 45 countries and increasing number of imported cases has been observed in Europe and United States. The aim of the study was to evaluated ZIKV epidemiology in travelers recently returning from endemic areas to Piemonte, Italy North-West (4.2 million inhabitants) from January to May 2016.

Methods: 68 samples (49 sera, 19 urine) were collected from 41 travelers returning from ZIKV endemic areas and referring to the regional Centre for Infectious Diseases, Amedeo di Savoia Hospital, Turin. Patients underwent laboratory examinations to rule out a tropical fever. Specific IgG and IgM antibodies to ZIKV were detected with ELISA IgM and IgG assay (Euroimmun, AG). Confirmatory Plaque Reduction Neutralization Tests (PRNTs) were performed at the Istituto Superiore di Sanità, Rome, Italy. Real Time Polymerase Chain Reaction (RT-PCR) for ZIKV was performed on serum/urine with two commercial assays: RealStar Zika virus RT-PC Kit (Altona Diagnostics) and Genesig Standard Kit (Primerdesign) validated with a ZIKV PCR standard (MR766 Zika virus strain) kindly provided by the Robert Koch Institute using 10-fold serial dilutions from 10^6 to 1 copy/μl.

Results: Recent ZIKV infection was identified in 3 out of 41 (7.3%) travelers. Patient 1 (male, 29 years old) reported arthralgia, retro-orbital pain, severe itching and mild burning maculopapular rash 5 days after returning from Venezuela. Leucopenia was present. Serology for ZIKV was IgM positive and IgG negative. ZIKV RNA was not detected in blood (urine not available). A second serologic test performed 2 months later showed IgG seroconversion (169 RU/mL) and undetectable IgM. Patient 2 (female, 31 years old) 2 days after returning from Venezuela, reported chest and limbs papular rash with arthralgia but no fever. ZIKV serologic tests showed a low IgG titer (50 RU/mL) with undetectable IgM. ZIKV RNA was negative in blood (urine not available). A second serum sample was withdrawn 2 months later and showed increasing IgG titer to 250 RU/mL. PRNTs for Patient 1 and 2 were positive for ZIKV neutralizing antibodies (titer ≥ 1:10). Patient 3 (female, 40 years old) 3 days after returning from the Dominican Republic presented with chest and limbs pruriginous rash and fever lasting from 8 days before, with leucopenia. ZIKV RNA was detected in urine (5913 copies/ml); in serum a high IgG titer (106 RU/mL) with undetectable IgM was reported. Nine days later, ZIKV RNA was still positive in urine (310 copies/ml); IgG titer increased to 265 RU/mL. All patients tested negative for Dengue and Chikungunya viruses and completely recovered after few days. ZIKV RT-PCR detection limit was 46 copies/μL for the Altona assay and 23 for Genesig [mean cycle threshold (Ct) values 37.3 and 37.5, respectively] according to the ZIKV MR766 standard.

Conclusions: Returning travellers are sentinels of a rapidly changing epidemiology and require a prompt diagnosis and a careful surveillance for their implications in subsequent autochthonous transmission of the disease. In this contest, standardized molecular and serologic tests are mandatory for the appropriate diagnosis.

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Abstract no: 164
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Screening of emerging viral infections among risk groups

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Background: Among viral infections, Hantaviruses and West Nile viruses (WNV) have been detected. Chikungunya virus, which can cause outbreaks in the temperate region, has been found in Italy. The members of Sandfly fever viruses (Genus: *Phlebovirus*): Sicilian, Naples, Toscana and Cyprus types have been detected in Italy, Portugal, Spain, France, Greece, Austria, Croatia and Turkey. Crimean-Congo hemorrhagic fever has been detected in the Balkan states, and dengue fever in Croatia, France and Norway. *Aedes albopictus*, the vector of yellow fever, is widespread among the European coastal regions and islands. The history of yellow fever and dengue fever in temperate regions confirms that the transmission of both diseases could recur.

Patients and Methods: PCR and RT-PCR (TIB Molbiol, Berlin, Germany and Roche, Mannheim, Germany) methods have been introduced for the screening of the nucleic acids of Chikungunya, Crimean-Congo hemorrhagic fever, Dengue, Hanta, Sandfly fever, and West Nile viruses in healthy risk groups (hunters, fishers, gardeners and keepers in zoological garden) and blood donors in South Hungary. Indirect immunofluorescence (IIF) methods were performed with BIOCHIP slides (Euroimmun Med. Lab. AG., Lübeck, Germany).

Results: PCR examinations proved negative both in risk groups and controls. Hantavirus Seul, Dobrava, and Puumala IgG antibodies proved positive in the cases of 5, 4 and 1 individuals, respectively. Sandfly fever viruses: Sicilian, Naples, Toscana and Cypres IgM were positive in 6, 2, 5 and 1,and IgG antibodies in 21, 17, 16 and 11 individuals. WNV IgM was positive in 3, and IgG in 22 cases. Chikungunya and Crimean-Congo IgM and IgG were negative. Dengue virus IgM was positive in 10 cases, while IgG was negative.

Conclusions: The intensification of migration, the growth in the density of the population, the susceptibility to infectious diseases, the decline of human immunity in consequence of insolation (UV effect), and malnutrition all tend to make humanity sensitive to infectious illnesses. Prevention, recognition, early diagnosis and treatment are very important to counter local endemics and epidemics.

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Abstract no: 173
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RIDA®GENE Zika Virus: A new commercial real-time RT-PCR assay for sensitive and reliable detection of zika virus in urine and serum samples

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Background: Besides endemic areas such as Africa and South East Asia, high numbers of zika virus infections were recently reported in South America, particularly in Brazil. In February 2016, the World Health Organisation (WHO) issued a Public Health Emergency of International Concern since microcephaly and other neurological disorders were increasingly reported in newborns of pregnant women with Zika virus infections. Zika virus belongs to the genus of *Flavivirus* and similarly to other members of the *Flavivirus* genus, transmission of zika virus occurs via mosquitos, in particular mosquitos of the *genus* Aedes. Cross reactions with other *Flaviviruses*, such as dengue virus or chickungunya virus, are often observed upon antibody-specific diagnostic testing so that confirmatory testing is required, in particular in areas where there have been possible co-infections.

Real-time PCR is a suitable method to specifically detect zika virus RNA within the first week after onset of symptoms in serum samples and in urine samples within 14 days after onset of symptoms.

This study aimed to evaluate a new real-time PCR assay for the detection of zika virus in urine and serum samples.

Materials and methods: The RIDA®GENE Zika virusreal-time RT-PCR assay detects zika virus-specific RNA by targeting the NS2A gene. An internal control RNA detects PCR inhibition, monitors reagent integrity and confirms successful nucleic acid extraction. The analytical reactivity and analytical specificity of the RIDA®GENE Zika Virus was tested using known quality control standards and reference materials. Spike experiments were carried out to determine the analytical sensitivity. A clinical evaluation of known positive and negative urine and serum samples was carried out on the LightCycler® 480II (Roche) and compared to two other commercially available test systems.

Results: Clinical evaluation of the RIDA®GENE Zika Virus with known positive and known negative serum and urine samples showed concurrent results when compared to two other commercially available test systems. Commercially available zika virus strains were used for evaluation of the analytical reactivity of the RIDA®GENE Zika Virus assay. No cross-reactivity to other *Flaviviruses* including dengue virus, chickungunya virus and west nile virus was detected with the assay. An analytical sensitivity of 50 copies/reaction was achieved with the LightCycler® 480II/LC2.0, Mx3005P, Rotor-Gene Q, ABI7500, CFX96 and SmartCycler II real-time PCR instruments.

Conclusions: The RIDA®GENE ZikaVirus real-time RT-PCR assay is a sensitive and reliable assay for the detection of zika virus, including both African and Asian variants. The assay is highly specific for zika virus without known cross-reactivity to other *flaviviruses* such as dengue virus or chickungunya virus. The validation of different common real-time PCR instruments provides broad flexibility for use in the routine diagnostics laboratory.

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Toscana virus meningitis in Southwestern France

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Aims: Arboviruses are gaining more attention due to the increased number of cases in human host. Toscana virus (TOSV) is a member of *Bunyaviridae* and was first identified in 1971 from a *Phlebotomus* in central Italy. TOSV has a tropism for the central nervous system and thus is recognized as an etiologic agent of meningitis in the areas where it is present. The virus circulates in the Mediterranean basin and during the warm seasons it might represent one of the main causes of acute viral meningitis.

In France, the first cases were described among German travellers returning from South East area in 1997 and the virus seems to be present on a large Mediterranean coastal zone.

Also, in Southwestern France the vector *Phlebotomus* is circulating as evidenced by the presence of numerous canine Leishmaniosis cases. But the incidence of aseptic meningitis linked to a TOSV infection has never been studied in this area.

Material and methods: Patients suffering from aseptic meningitis without any documented etiology were matched on sex and age with patients suffering from documented enteroviral meningitis ($n = 58$). All patients were attending, depending on age, children or adults Toulouse University Hospital emergency unit.

We looked for Toscana virus, Herpes simplex virus 1 and 2 (HSV1-2) and Human herpes virus 6 (HHV6). Nucleic acids (NA) were extracted from cerebro-spinal fluid (CSF) (samples stored at −20 °C pending batch analysis) with the MagNA Pure 96 DNA and Viral NA Small Volume kit® on the MagNA Pure 96™ instrument (Roche Molecular Diagnostics, Meylan, France) (input volume: 200 μl, output volume: 100 μl) according to the manufacturer's instructions.

Extracted NA were tested employing a monoplex in-house RT-PCR for HSV1-2 and HHV6 on the Light Cycler (Roche Molecular Diagnostics, Meylan, France). TOSV was tested using the CFX96™ Real-Time System (Biorad diagnostics).

Results: We tested 66 CSF sampled from mid-april to mid-october in 2014 and from mid-april to mid-october in 2015 among patients (sex ratio M/F: 1.3) suffering from aseptic acute meningitis negative for enterovirus. 21 patients were aged 5–10 year-old, 16 were 11–20, 16 were 21–30, 10 were 31–41 and 3 were older than 41 year-old.

No sample was positive for HSV1-2 and HHV6.

We detected however one sample positive for TOSV in a 36 year-old woman's CSF. She was suffering from a classical form of aseptic meningitis (fever, headache and vomiting) associated to myalgia but without photophobia and was discharged after three days of hospitalization with favorable outcome.

Conclusion: This case is the first case of detection of Toscana virus in the Southwestern France and demonstrates that this virus could be a causative agent for aseptic meningitis in this area.

Furthermore our results showed that HSV1-2 and HHV-6 are very rarely the causative agents of meningitis among patients older than five year-old.

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From TYPENED to REGIOtype to EUROtype: Moving towards a comprehensive surveillance strategy for emerging viruses

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Background: Patients with respiratory illness are screened for viral infections. This screening was expanded by routinely sequencing enteroviruses and rhinoviruses to identify outbreaks, new viral strains and transmission patterns. Additionally, we included noroviruses and parechoviruses in patients with gastro-intestinal complaints or signs of encephalitis/meningitis. For surveillance purposes, a national structure called TYPENED is available through the Dutch National Institute of Health (RIVM). Our main objective is to provide a rapid regional sequencing service that create a source of clinical information and to correlate this with epidemiological patterns.

Methods: A sequencing and epidemiological strategy for surveillance, called REGIOtype, was implemented by including GP's, regional hospitals and regional Public Health Services which covers the Northern part of the Netherlands, for a rapid flow of information within our region. Sequencing is performed locally and results are available within one week. Clinical data are exchanged and phylogenetic analysis is performed.

Results: Phylogenetic analysis has contributed to the knowledge of circulating viral strains and the relatedness within and between multiple institutions. In total 2629 samples were processed for sequencing within a 2 years period. 2223 of these samples (85%) were successfully sequenced, the other 406 (15%) failed, mainly due to a low viral load. 443/2223 (20%) were enterovirus, 891 (40%) rhinovirus, 768 (35%) norovirus and 121 (5%) parechovirus. In total, 962/2223 (43%) sequences were obtained from healthcare institutions in the region and 1261 (57%) from the university hospital. Enterovirus D68 and recently the 'new' C-group enteroviruses were identified using this strategy as well as a vaccine derived poliovirus strain from a refugee.

This rapid sequencing strategy in combination with the clinical data enables the application of infection control measures in the participating (local) health institutions. Besides, these data not only indicated a possible outbreak, but it also improved detection methodologies in our regional laboratories.

Conclusion: REGIOtype has proven to be an adequate strategy for surveillance on local circulating viruses and not only for the hospitalized population with severe (underlying) illnesses. This underlines the importance for regional surveillance by sequencing, of which several networks are available within Europe but still acting independently from each other.

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Abstract no: 294
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Multicenter investigation of bufavirus in the etiology of viral central nervous system infections of adults and children

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Introduction: Bufavirus (BuV) is a newly-identified parvovirus, detected in the diarrhoeal stool samples of patients from Burkina Faso. The global distribution, epidemiology and genetic characteristics of BuVs infections are obscure. Considered primarily as an agent causing gastroenteritis, the association and outcome of BuV infections with various clinical presentations mostly remain explored. This study was undertaken to investigate probable impact of BuV in central nervous system infections in a region where it was previously reported to cause human infections

Methods: The study was undertaken in 3 institutions in Ankara province, Central Anatolia, Turkey. Patients, clinically diagnosed with febrile disease and/or central nervous system infections of presumed viral aetiology, were enrolled in the study with informed consent. Sera and/or cerebrospinal fluid specimens were collected from 95 children attended to Gazi University Hospital and Diskapi Training and Education Hospital from during 2011–2013 and 38 adult patients, attended to Hacettepe University Hospital from June 2012 to March 2013. Clinical history and follow-up, physical examination and standard laboratory findings of the patients were recorded. Nucleic acid extraction was performed via commercially available spin-column assays and BuV detection was carried out by in house nested-PCR utilising previously-described primers.

Results and conclusion: In all patients, bacterial, mycobacterial and fungal cultures were negative, as well as PCR for herpes simplex virus (HSV) types 1/2. PCR results of all samples were negative for BuV. This is the first study that evaluates a probable association of BuV and central nervous system infections. Although

Parvovirus B19, a well-characterized human pathogen can rarely cause encephalitis, our findings did not confirm such an association for BuV in this preliminary investigation. However, long-term evaluation of individual cases with unknown etiology is required and might reveal this virus to involved in certain settings.

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Abstract no: 330
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Mumps outbreak among vaccinated students in Trondheim, Norway in 2015

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Background: Mumps vaccination (genotype A) was introduced in the Norwegian childhood vaccination schedule in 1983 with vaccination coverage exceeding 90%. At the end of August 2015, a foreign student was hospitalized at St. Olavs Hospital in Trondheim with parotitis and orchitis, and during the period September 2015 to January 2016, 176 suspected cases were registered within the municipality of Trondheim.

Material and methods: Diagnosis was confirmed by an in-house PCR, viral culture and/or serology. Specimens from the oral cavity was collected by flocked swabs in UTM-medium (Copan), and was the method of choice. Isolates were sent to Norwegian Institute of Public Health in Oslo for genotyping.

Results: Of 148 confirmed cases, 127 were students, and the vast majority had been vaccinated against mumps. Seven patients were hospitalized, six had orchitis and one patient had meningitis. The health authorities in Trondheim vaccinated close contacts and unvaccinated students. 53 isolates were genotyped, and they were all genotype G. Mumps PCR was positive until 11 days after onset of symptoms. Furthermore, the virus could be cultured until 9 days after symptom debut. The sensitivity of nasopharyngeal and urine specimens was too low to be used for diagnostic purposes. EBV-DNA was detected in 13 of 27 (47%) specimens testing positive for mumps virus RNA, but in low concentration. Viral culture proved to be important for confirmation of the first cases, and to adjust the sensitivity of the in-house PCR. Only one (5%) out of 20 vaccinated students with confirmed mumps infection had detectable serum IgM using the LIAISON® Mumps IgM assay.

Conclusion: The outbreak of mumps among vaccinated students suggests that the current mump vaccines may not be effective in preventing genotype G mumps outbreaks. Serological methods often fail to detect mumps infections in immunized patients, and PCR from specimens taken from the oral cavity is the test of choice to diagnose these infections.

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Analytical performance and method comparison of the VERSANT Zika RNA 1.0 Assay (kPCR)

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Background: Zika virus (ZIKV) is a mosquito-borne virus of the family Flaviviridae first isolated in 1947 in Uganda. The first ZIKV outbreak outside of Africa and Asia occurred in 2007 in Yap Island (Federated States of Micronesia). The largest outbreak was from October 2013 to March 2014 in French Polynesia (FP), Pacific. The World Health organization (WHO) recently declared that the ongoing recent cluster of microcephaly cases and other neurological disorders reported in the Americas, constitutes a Public Health Emergency of International Concern (PHEIC) [1].

We present here analytical studies and a method comparison with clinical samples of a qualitative diagnostic real-time PCR assay, the VERSANT® Zika RNA 1.0 Assay (kPCR) [2].

Method: The VERSANT Zika RNA 1.0 Assay (kPCR) qualitatively detects ZIKV RNA. ZIKV RNA from plasma or serum is extracted using the VERSANT Molecular Prep SP with VERSANT Sample Preparation 1.0 Reagents Kit and then amplified on the Thermo Fisher QuantStudio 5 thermal cycler, Bio-Rad CFX96 Real-Time PCR Detection System, or the Applied Biosystems 7500 Real-time PCR System. Two amplification reactions, targeted to portions of the NS2 and NS5 regions of ZIKV, comprise the assay.

Inclusivity of the assay was evaluated *in silico* comparing primer/probe sequences against 35 unique Zika sequences from 14 countries. Assay specificity was tested with 50 individual ZIKV-negative clinical plasma specimens. Cross-reactivity of the assay was evaluated with high titer inactivated pathogens: Dengue (strains 1–4), Yellow Fever 17D, Chikungunya, West Nile, Human Parvovirus B19, and Mayaro viruses, as well as protozoan *Plasmodium falciparum*. An additional 51 organisms were evaluated *in silico*. Analytical sensitivity of the assay was evaluated using a dilution series of ZIKV (Zeptometrix, strain PRVABC59) with concentration determined by a $TCID_{50}$ endpoint dilution assay. A method comparison with a CDC assay using primers/probe from Lanciotti et al (2007) was conducted on 90 clinical plasma or serum specimens suspected by a physician or confirmed by home brew assay to contain ZIKV.

Results: All 35 of the published ZIKV strain sequences showed 100% homology with at least one of the two amplification reactions. No amplification was observed in ZIKV-negative clinical specimens. No cross-reactivity was observed with any of the tested pathogens tested and no significant sequence homology was found for any of the 51 organisms evaluated *in silico*. An assay limit of detection of at least 0.05 U/mL ($TCID_{50}$) was established in both plasma and serum on each of the three thermal cyclers. In the method comparison, both assays detected ZIKV in each of 34 specimens and did not detect ZIKV in each of 44 specimens. The VERSANT Zika RNA 1.0 Assay (kPCR) detected ZIKV in an additional 10 specimens that the comparator CDC assay did not, while the comparator CDC assay detected ZIKV in 2 specimens that VERSANT Zika RNA 1.0 Assay (kPCR) did not.

Conclusion: The VERSANT Zika RNA 1.0 Assay (kPCR) qualitatively detects ZIKV RNA. This assay recognizes a broad spectrum of published ZIKV RNAs in silico, has high analytical sensitivity, is specific to ZIKV among the family Flaviviridae viruses, and has excellent performance with clinical specimens.

Reference

[1] World Health Organization, Zika Virus Fact Sheets, 2016.
[2] Z. Versant, ika RNA 1.0 Assay (kPCR) under feasibility evaluation. Not for sale, and its future availability cannot be guaranteed, 2016 (in press).

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Molecular epidemiology of enterovirus in Scotland, January 2013–December 2015

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Introduction: Enteroviruses are common viruses which cause a variety of symptoms ranging from mild illness, such as fever, rash, and cold-like symptoms, to more severe conditions, such as viral meningitis or encephalitis. Non-polio enteroviruses (EV) are a group of ssRNA viruses with over 100 different serotypes. They are the major aetiological agent of childhood meningitis as well as, rarely, encephalitis and acute flaccid paralysis [1,2].

Methods: In-house PCR is used for the detection of enteroviruses (including polioviruses) and parechoviruses in faeces, throat swabs, CSF and blood specimens. All significant enterovirus or parechovirus positive results (i.e. detected in CSF or with significant symptoms) are recommended to be submitted for typing. All EV PCR positive CSF, faecal and throat swab isolates were typed by sequencing of VP1 or VP4 [3]. Epidemiological information and typing data were compared for the specimens received in the 24 month period between 1st January 2013 and 31st December 2015.

Results: A total of 329 specimens, representing 315 patients, were submitted to the laboratory for typing between 1st January 2013 and 31st December 2015. The majority of samples were cerebrospinal fluid samples (66%) or throat swabs (19%). Age and gender data was available for >99% of the cases. Thirty-nine percent of the cases were female. Median age of patients was 1 month (range: 16 days–81 years). Interestingly, half of the samples were from patients <1 year old. Typing results were available for 80% of all samples. Twenty-seven different EV serotypes were detected. The commonest enterovirus types were coxsackievirus B5 (12.8%), echovirus 6 (12.1%), echovirus 30 (5.5%), coxsackievirus A6 (5.5%) and Echovirus 9 (4.0%). Distinct seasonality of enterovirus was observed with peaks of infection occurring in September 2013, June 2014 and October 2015. Most notable was the predominance of Echovirus 6 and Coxsackievirus B5 in autumn 2015.

Discussion: EV surveillance is important not only for monitoring the changing epidemiology of these infections but also for the rapid identification of spread of emerging EV. In Europe within the last decade, echovirus 30 was the cause of the majority of outbreaks associated with CNS infections [4]. In 24 months of enterovirus typing in Scotland we have identified peaks of infection predominated by echovirus 6 and coxsackievirus B5 in autumn 2015.

Reference

[1] H. Harvala, N. McLeish, J. Kondracka, C.L. McIntyre, E.C. McWilliam Leitch, K. Templeton, et al., Comparison of human parechovirus and enterovirus detection frequencies in cerebrospinal fluid samples collected over a 5-year period in Edinburgh: HPeV type 3 identified as the most common picornavirus type, J. Med. Virol. 83 (5) (2011) 889–896, http://dx.doi.org/10.1002/jmv.22023, PMID: 21412796.
[2] H. Rudolph, H. Schroten, T. Tenenbaum, Enterovirus infections of the central nervous system in children: an update, Ped. Inf. Dis. J. 35 (5) (2016) 567–569.
[3] S. Bennett, H. Harvala, J. Witteveldt, E.C. McWilliam Leitch, N. McLeish, et al., Rapid simultaneous detection of enterovirus and parechovirus RNAs in clinical samples by one-step real-time reverse transcription-PCR assay, J. Clin. Microbiol. 49 (7) (2011) 2620–2624.
[4] A. Nougairede, M. Bessaud, S.D. Thiberville, et al., Widespread circulation of a new echovirus 30 variant causing aseptic meningitis and non-specific viral illness, South-East France, 2013, J. Clin. Virol. 61 (2014) 118–124.

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Abstract no: 353
Presentation at ESCV 2016: Poster 77

Ebola virus outbreak in West Africa – Portuguese laboratory response overview

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The Ebola outbreak in West Africa was the largest and most complex outbreak since the virus was discovered in 1976. First cases were notified in March of 2014 and the last ones were reported in April of 2016 in Liberia.

To respond to the epidemic of Ebola virus, Portugal created an coordination committee where the National Institute of Health, through the Emergency Response and Biopreparedness Unit (UREB), participated integrating the "Platform Response to Ebola Virus Disease".

This unit is the national reference laboratory for biological events or catastrophes and has skilled professionals, know-how, BSL-3 facilities, capacity to work 24 h/7 d and trained human resources to increase lab capacity in emergency situations. The laboratory diagnosis capacity includes the detection of bacteria, virus and toxins, which are considered bioterrorism agents, using Microbiology, Immunology and Molecular Biology techniques. In order to ensure quick and reliable results, a laboratory algorithm was developed taking in account the available human and technical resources. UREB also participates regularly in International External Quality Assessments, training courses and simulation exercises.

Although Portugal does not have a BSL-4 facility, the participation in European projects as QUANDHIP, allowed the upgrade of Biosafety procedures, technical skills and the use of a glove container for samples inactivation permitting the analysis of suspected samples, avoiding the need to send suspected samples to abroad.

In Portugal 15 samples from suspected cases concerning patients who were traveling from African countries were received at UREB. All samples were negative for Ebola virus, and the differential diagnosis was performed in parallel which includes the detection of *Plasmodium* spp., Marburg and Lassa virus. Forty percent of suspected cases were positive for *Plasmodium falciparum*.

The algorithm of laboratory procedures for samples suspected to Ebola virus it was well implemented and was several times tested through the participation in simulation exercises. The communication of the results to the competent authorities occurred in 4–5 h from the reception of the sample in the laboratory.

The experience gained and work accomplished enabled a quick and effective laboratory response and permitted to increase training actions, BSL-3 facility upgrading, development of national

guidelines and establish an agreement with European reference BSL-4 laboratories for additional tests.

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Abstract no: 111
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Norovirus genotypes diversity in sporadic cases and in outbreaks of acute gastroenteritis in Spain: A 10-year study

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Introduction: Human norovirus (NoV) is a major cause of viral acute gastroenteritis worldwide. NoV causes sporadic cases and outbreaks of acute gastroenteritis (AGE), often in health care settings (including hospitals and elderly care homes), where the virus is predominantly spread from person to person. Most human NoVs belong to genogroups GI and GII, with more than 30 genotypes. The transition of endemic to epidemic NoV genotypes remains poorly understood. Despite the great genetic diversity among NoVs, a single genotype, GII.4, further subdivided into 'variants', is responsible for the majority of the NoV outbreaks worldwide. The aim of this study was to analyse the temporal distribution and variation of NoV strains causing sporadic cases and outbreaks of AGE during a decade, taking into account that NoV infections are clearly underdiagnosed.

Materials and methods: A total of 1610 stool samples from sporadic cases and outbreaks of AGE were analysed by RT-PCR targeting the RNA polymerase with primers JV12/JV13 and eventually with primers G1SKF/G1SKR or G2SKF/G2SKR for capsid gene amplification. RNA was extracted from stool samples with Trizol reagent (Life Technologies). NoV was detected by ORF1 (polymerase) and/or ORF2 (capsid gene) RT-PCR. Genotyping was carried out by sequencing PCR amplicons, 114 obtained from outbreak specimens and 253 from sporadic case specimens. Genotypes were identified by using the BLAST program and by the NoV automated genotyping tool (http://www.rivm.nl/mpf/norovirus/typingtool).

Results: NoV strains causing outbreaks along the 10 years analysed in this study have evolved in a sequential manner, starting in 2006 with GII.4 Den Haag_2006b variant, followed thereafter by GII.4 New_Orleans 2009 and by GII.4 Sydney 2012. In the last year a new genotype, GII.17, has arisen for the first time causing outbreaks. Sporadic cases of NoV gastroenteritis were caused by the same strains during their circulation periods, as well as by many other genotypes regarded as non-epidemic: GI.1, GI.2, GI.3, GI.4, GI.7, GI.9; GII.1, GII.2, GII.3, GII.4 Apeldoorn 2007, GII.4 2010, GII.7, GII.12, GII.13, GII.16, GII.17, GII.21 and GIV.

Conclusions: The temporal distribution of NoV genotypes is very dynamic and difficult to predict according to the historical data recorded by our laboratory and by others. Norovirus genotype surveillance is necessary to detect the emergence of new strains.

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Abstract no: 130
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Detection and characterisation of viral pathogens causing gastroenteritis in Ireland, 2014–2016

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Background: The National Virus Reference Laboratory (NVRL) receives ~12,000 faecal samples a year from patients presenting with suspected viral gastroenteritis (VGE) in Ireland. All samples are tested for norovirus, rotavirus, sapovirus, astrovirus and enteric adenovirus. We present two years data from the VGE screen. Rotavirus vaccination will be included in the national infant immunisation schedule in Ireland from the 1st October 2016. The NVRL offers a rotavirus genotyping service and the data generated will determine a baseline of circulating strains in Ireland and will monitor the genetic evolution post implementation of vaccination. The NVRL also contributes to the surveillance of norovirus by genetically characterising circulating strains to monitor the changing epidemiology of the viral genotypes.

Methods: An in-house real time PCR was developed and validated to detect the five main causes VGE. The genotyping method was a WHO PCR method of the VP7 and VP4 regions and electrophoresis to determine the G and P genotype. The genetic analysis of the norovirus was performed using Sanger sequencing.

Results: Seasonal trends of the five viruses were observed as expected. In 2015, circulation of viruses associated with VGE peaked in February and March. In February 2015 the NVRL received 1218 samples for testing. Of these 239 (20%) were positive for norovirus and 146 (12%) were positive for rotavirus. In March 2015, 1404 samples were received; 213 (15%) and 183 (13%) were positive for norovirus and rotavirus respectively. In 2016 the VGE season peaked a month later than in 2015. In March 2016, 1366 samples were tested and were norovirus positive in 277 (20%) of samples and rotavirus in 63 (5%) of samples. In April 2016, 1416 samples were tested and 247 (17%) and 115 (8%) were detected as norovirus and rotavirus respectively. The remaining viruses were detected at lower levels; sapovirus and astrovirus were more dominant in the winter months whereas enteric adenovirus demonstrated no obvious seasonal trend. Approximately 3% of the positive samples contained more than one virus, the most common combination being norovirus and rotavirus. In addition to norovirus causing outbreaks of VGE, we found that rotavirus and sapovirus have been responsible for outbreaks within the paediatric and elderly population. The commonest strain of norovirus circulating was Norovirus GII which accounted for 90% of norovirus cases. Phylogenetic analysis of a representative number of norovirus outbreaks and sporadic cases performed in 2014/2015 season, identified viruses clustering with GI.3, GII.1 GII.3, GII.4 and GII.6 strains. The recently described new variant of GII.17 was not detected in this season. To date 52 rotavirus positive samples have been genotyped. The most commonly detected strains were G9P[8] (40%) and G1P[8] (33%); followed by G4P[8] and G2P[4] (19% and 8% respectively).

Conclusion: The predominant virus detected in suspected cases of VGE was norovirus in both adults and children. This was followed by rotavirus, sapovirus, astrovirus and then enteric adenovirus. The rotavirus genotyping results indicate that the rotavirus strains detected in Ireland reflect those that are circulating in other European countries. Interestingly there appears to be larger proportion of G9P[8] in Ireland than reported in other countries. As in previous

years for norovirus, the 2014/2015 season showed the GII.4 (GII.4 Sydney 2012), remained the prevalent circulating strain.

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Abstract no: 154
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Phylogenetic analysis of G12 group A rotavirus circulating in Spain during 2012–2015: Detection of different clusters with distinct evolutionary origins

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Introduction: G12 group A rotavirus (RVA) is currently regarded as a common genotype in many geographical areas, in combination with several P-types. Rotavirus strains emerging in Europe in the last 10 years include G8, G12 and G3P[8] genotypes. Analysis of the different patterns of emergence for G12 strains has already been published [1]. In Spain, G12P[8] RVA strains were described for the first time in the Basque country in 2004–2005, being the predominant genotype during the 2010–2011 rotavirus season. Since then, an increase of G12P[8] strains was detected in other Spanish regions (Castilla-León, Aragón, Catalonia and Valencia), accounting for 27.5% of all rotavirus-positive samples.

Objective: To analyse phylogenetically the G12 rotavirus strains detected in different areas of Spain (Valencia, Zaragoza, Valladolid and Barcelona) during the last seasons, in order to evaluate their genetic variability and to compare their antigenic sites in VP7 and in VP8* (VP4) with those of reference and vaccine strains.

Materials and methods: Viral dsRNA was extracted from 61 fecal samples using Trizol reagent. The G and P types were analysed by RT-PCR following standardized procedures (http://www.eurorota.net/docs.php). Nucleotide sequencing of VP7 (nt 88-876) and VP4 (nt 88-876) amplicons was performed. Phylogenetic analyses were performed using the MEGA6 software (www.megasoftwares.com). Trees were constructed using the maximum likelihood method and the Tamura-3 parameter as a nucleotide substitution model with the statistical support of 1000 bootstrap repetitions.

Results: Phylogenetic analyses of the VP7 and VP4 genes demonstrated that they belong to lineages III of both G and P types. These strains display the typical human Wa-like gene constellation, and this may be the key to their recent increase and spread. Fifty-five G12 stains were associated with P[8] and six with P[6]. Both VP7 and VP4 (VP8*) genes could be separated into two different clusters, with one of the VP4 (VP8*) clusters subdivided into two subgroups. Antigenic regions were more conserved in VP7 than in VP4 (VP8*). However, more diversity was found in the antigenic sites of VP7 when compared with the RVA vaccine strains. Our results suggest different evolutionary origins for each genetic cluster.

Conclusions: The transmission and genetic evolution of rotavirus strains depend on many factors, including biological fitness, resistance to environmental stress and pre-existing immunity in the population. In particular, VP7 and VP4 antigenic region variations may play an important role in the ability of RVA to escape to the immune response. G12P[8] strains may have encounter all these circumstances allowing their infectivity and rapid spread.

Reference

[1] Iturriza-Gómara, et al., Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network, Epidemiol. Infect. 139 (2011) 895–909.

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Abstract no: 16
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Detection of adenovirus in diarrheal children between 0 and 5 years old and except adenovirus serotype 40/41 by DNA sequencing, and phylogenetic analysis

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Background: Adenovirus is one of the most important factors in children with acute gastroenteritis between the 0 and 5 years old all over the world. In this study we aimed to determine the frequency of adenovirus that is one of the viral gastroenteritis agents in children between 0 and 5 years old and investigate the distribution according to clinical findings, age groups, months and seasons.

Materials and methods: Stool samples were obtained from 180 children of 0–5 years old with acute gastroenteritis attended between July 2007 and June 2011 to the Ankara Training and Education Hospital. Stool samples were analysed by rapid chromatographic immune diagnostic test, enzyme immune assay (EIA) and polymerase chain reaction (PCR). These methods that used in the diagnosis of adenovirus were compared with each other and discussed of the advantages of the methods. Samples were analysed with hexon gene specific primers by PCR and DNA sequence analysis and identified adenovirus types associated with gastroenteritis outside 40/41. Phylogenetic analysis was made and adenoviruses that have seen in our city were evaluated.

Results: The samples were found to be positive 5%(9/180) by immune chromatographic method; 6.1%(11/180) by EIA; 13.9%(25/180) by PCR. Adenovirus gastroenteritis did not show any difference in age group, gender, month and season. We identified that vomiting in adenovirus gastroenteritis is an important finding, but not common clinical table in the adenovirus gastroenteritis. Most of the children that seen adenovirus gastroenteritis is with diarrhoea and daily diarrhoea were found to be 6 and above were observed. Compared to PCR, the sensitivity of the immune chromatographic method was 36% and specificity was 100%, PPV was 100% NPD was 90.6%; EIA test sensitivity has been identified as 44%, specificity 100%, PPV 100%, NPV 91.7%. In our study, 25 samples were found to be positive by PCR, 16(64%) for positive AdV41; 6(24%) for positive AdV40, 2(8%) for positive AdV31, 1(4%) for positive AdV7.

Conclusion: As a result of our work, it is shown that AdV31 and AdV7 can be associated with gastroenteritis with AdV40/41 serotypes. Highest frequency of adenovirus serotypes was 64% with AdV41. In this study, genotyping and phylogenetic analysis of enteric adenoviruses have been made for the first time in our country. Adenovirus serotypes showed similarity with Asian and American serotypes, 80% (20/25) and 20% (5/25) respectively. Adenovirus serotypes that detected in our study were in concord-

ance with Asian serotypes; however, two samples were detected as serotype 31 which is endemic for USA.

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Abstract no: 2
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Bufavirus genotype 3 in Turkish children with severe diarrhoea

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Introduction: Recently a parvovirus called bufavirus (BuV) has been implicated as a causative agent of diarrhoea. Every year, a substantial number of children are affected by viral diarrhoea in Turkey. However the role of emerging viruses in Turkish children with diarrhoea has not been widely investigated. To further reveal the epidemiology, pathogenic role and genetic characteristics of BuV, this study was performed in Turkish children with diarrhoea.

Materials and methods: From September 2004 through June 2011, 1221 diarrheal stool samples were collected from children under 5 years of age attended at the Gazi University Hospital and Ministry of Health Ankara Training and Education Hospital, Ankara, Turkey. All samples were tested for pathogenic bacteria, parasites, rotavirus and norovirus (NoV). Excluding the ELISA positive rotavirus and NoV samples, 583 samples were available for BuV detection. From February through September 2013, 148 normal stool samples were collected from children attended at the well child care clinic of the Dept. of Paediatrics, Gazi University. These children attended the clinic for routine immunization and developmental check-up and had no diarrhoea for the last one week. DNA extraction was done by using spin-column method (QIAamp Viral RNA Mini Kit, Qiagen, Germany) and bufavirus amplification was carried out by in house PCR (Promega, ABD). The nucleotide sequence of the concerned genes were determined by BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) according to the instructions of the manufacturer and the product was run into ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Rotavirus, adenovirus, human bocavirus (HBoV), astrovirus, NoV, salivirus, cosavirus and Aichi virus were tested for in the BuV-positive samples by in house PCR.

Results: All samples were negative for diarrhoea causing bacteria or parasites. BuV was detected in 8 (1.4%) samples with diarrhoea. Age and gender were not statistically significant for bufavirus detection. Positive stool samples were belong to years 2007, 2008 and 2010; however there was not any statistically significant difference between years. According to the other seasons in autumn bufavirus positivity was higher without statistically significant difference. Three samples were co-infected with NoV GII.21, NoV GII.4 and human bocavirus (HBoV)2, and HBoV3. All stool samples from healthy children were negative for BuV. The number of diarrhoea in BuV positive patients was significantly more than in other diarrheal patients ($p = 0.017$). Phylogenetic analysis of the VP1 gene showed that Turkish strains were in close association with Bhutanese BuVs and belongs to genotype 3. The NS1, VP1, and VP2 of the Turkish strains showed close nucleotide and amino acid identities among themselves and with Bhutanese strains. The frequency of tandem repeats in the 3′ untranslated region of Turkish BuVs was different than Bhutanese BuVs.

Conclusions: Absence of BuV in the stool of normal children and association of BuV in children with diarrhoea may support the pathogenic role of BuV. BuV associated diarrhoea was severe in Turkish children. BuV3 is possibly prevalent in Asian countries.

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Abstract no: 203
Presentation at ESCV 2016: Poster 83

Multiplex technology for the detection of gastrointestinal viruses in stool samples from diarrheic children

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Aims: To evaluate the multiplex FTD Viral Gastroenteritis® (Fast-Tracks diagnostics®) for the detection of 6 viruses in stools samples collected from diarrheic children. To compare the results with those obtained with the routine techniques for the detection of adenovirus and rotavirus.

Materials and methods: Stool specimens from 118 children and 51 neonates who were suffering from acute diarrhea were used to evaluate the FTD viral gastroenteritis®. This technique is a two tubes multiplex (tube 1: Norovirus G1, Norovirus G2 and Internal Control; tube 2: Adenovirus, Rotavirus, Astrovirus) plus add-on singleplex PCR for the detection of sapovirus.

Nucleic acids were extracted from native stools or swab with the MagNA Pure 96 DNA and Viral NA Small Volume kit® on the MagNA Pure 96™ instrument (Roche Molecular Diagnostics, Meylan, France). Nucleic acids were tested with the FTD viral gastroenteritis® according to the manufacturer's instructions on a CFX™ Instrument (Biorad diagnostics).

The results were compared with those obtained with the techniques used in routine for the detection of rotavirus and adenovirus, *i.e.* BIOSYNEX Adenovirus-Rotavirus Combo® (BIOSYNEX). All samples showing any adenovirus discrepancies were tested using an in-house real-time PCR method.

Data were analyzed using StataTM software (StataCorp, Texas). The match between the assays was assessed using the McNemar Chi-squared test. p values of less than 0.05 were considered significant.

Results: 188 stool samples were tested from 169 children (88 males). 118 (65 males) children (118 specimens) were attending the emergency care unit (mean age 2.80 – median 9 – range: 0–15) and 51 (23 males) children (70 specimens) were attending the neonatology unit (age under one).

83 pathogens were detected among the children attending the emergency care unit. The most commonly detected was Rotaviruses ($n = 70$, 84.3%), followed by adenovirus ($n = 8$, 9.6%) and astrovirus ($n = 3$, 3.6%). One sample was positive for norovirus G2 and one sample was positive for sapovirus. Four coinfections were detected involving rotavirus and adenovirus ($n = 2$) and rotavirus and astrovirus ($n = 2$).

Nine pathogens were detected among the neonates. The most commonly detected was rotavirus ($n = 4$, 44.4%), followed by sapovirus ($n = 4$, 44.4%). One sample was positive for adenovirus. One sample showed co-infection (rotavirus and sapovirus).

The FTD viral gastroenteritis® detected 13 more rotavirus ($p < 0.005$) than did the BIOSYNEX Adenovirus-Rotavirus Combo®. Nine samples were adenovirus positive with the FTD viral gastroenteritis® and were negative with the BIOSYNEX Adenovirus–Rotavirus, while three samples gave the opposite results. Tested with the adenovirus in-house real-time PCR, 6/9 samples were positive ($p < 0.05$), all the remaining three were negative.

Conclusion: The FTD viral gastroenteritis® is a very sensitive and convenient method for detecting multiple gastrointestinal virological pathogens.

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Abstract no: 208
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Comparison of rotavirus frequency and genotype distribution in Rwanda before and after vaccine introduction

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Acute gastroenteritis is a major reason of disease and death among children in developing countries. A wide range of viruses, bacteria and protozoa, with rotavirus as one of the most important pathogens, can cause infectious diarrhoea. In May 2012, rotavirus vaccination with pentavalent RotaTeq vaccine was introduced in the national immunization program in Rwanda. The vaccine coverage in children younger than 1 year of age was 98% in 2014 and the number of hospital admission for diarrhoea specific to rotavirus after vaccine introduction has recently been reported to decrease significantly. Still, the incidence and the genotype distribution of rotavirus infections in the community are not known. In this work we compared the frequency of rotavirus together with other diarrhoeagenic pathogens, and analysed rotavirus genotypes before and after the introduction of vaccination.

Faeces samples from children below five years of age, collected before vaccine introduction between 2010 and 2012 ($n = 829$) and after in 2014 ($n = 658$, 89% vaccinated), were analysed by real-time PCR targeting a wide range of pathogens. Rotavirus positive samples were genotyped by a real-time PCR assay targeting rotavirus VP7 (G1, G2, G3, G4, G9 and G12) and VP4 (P[4], P[6] and P[8]) to identify the most common genotypes.

The proportion of samples that were of rotavirus positive was 34% before vaccine introduction. In 2014, the rotavirus was detected in 30% of vaccinated and 27% of unvaccinated children. Interestingly, norovirus genogroup II, sapovirus and astrovirus were significantly ($p > 0.0001$) more common in 2014, after vaccine introduction. There were marked shifts in rotavirus genotypes over time: in 2010, G2P[4] was detected in 87%, G12P[6] in 13%; in 2011, G9P[8] was detected in 48%, G1P[8] in 30% and G12P[6] in 16%; in 2012, G9P[8] was detected in 68% and G12P[6] in 21%. In 2014, after vaccine introduction, G12P[8] was found 81% and G4P[8] in 19%, without difference between vaccinated and unvaccinated children.

The results show no difference in rotavirus frequency after the introduction of rotavirus vaccine into the immunisation program, and that rotavirus infection frequencies were similar in vaccinated and unvaccinated children. Marked changes of the rotavirus genotype distribution were observed from year to year also before the introduction of vaccination in 2012, and therefore genotype changes after that time point may not be due to vaccination itself.

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Abstract no: 230
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Evaluation of VIASURE real-time PCR assays for detection of rotavirus and norovirus GI and GII in fecal samples

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Introduction: Rotaviruses (RVs) and noroviruses (NoVs) are the main etiological agents of nonbacterial acute gastroenteritis (AGE) in both children and adults. A rapid and sensitive detection is crucial to implement measures to reduce the spread of gastroenteritis infections. We evaluated the efficacy of the VIASURE Rotavirus Real Time PCR Detection Kit, the VIASURE Norovirus GI Real Time PCR Detection Kit and the VIASURE NoV GII Real Time PCR Detection Kit (Certest Biotec) for their diagnostic application in a clinical laboratory. The sensitivity and specificity of the VIASURE Rotavirus assay was compared with those of the Rotavirus–Adenovirus immunochromatographic (ICG) test (Certest Biotec), an in-house conventional RT-PCR for rotavirus detection and the RIDA®GENE Viral Stool Panel II real-time RT-PCR (R-Biopharm AG). The performance of the VIASURE Norovirus GI and GII assays was also compared with an in-house conventional RT-PCR for norovirus detection and the RIDA®GENE Norovirus I & II real-time RT-PCR (R-Biopharm AG).

Materials and methods: Fecal samples from children and adults with AGE were collected and diluted in PBS to prepare 10% fecal extracts. The presence of RV was tested in 210 samples and NoV GI and GII were analyzed in 181 samples. RNA was extracted from 200 μl of fecal suspensions by using the VIASURE RNA/DNA Extraction Kit (Certest Biotec). The immunochromatographic (ICG) test for rapid RV detection was performed following the manufacturer's instructions. Conventional RV RT-PCR reactions for VP7, VP4 and/or VP6 viral genes were carried out according to the protocols described by the European Rotavirus Network (http://www.eurorota.net/). A conventional NoV RT-PCR was performed using primers JV12/JV13 targeting the polymerase gene and eventual capsid gene amplification. The real-time RT-PCR assays evaluated in this study were performed according to the manufacturer's instructions using the StepOne™ real-time PCR equipment (Applied Biosystems) for the VIASURE kits and the ABI 7500 real-time PCR System (Applied Biosystems) for the RIDAGENE kits. Samples with a Ct value ≤35 were considered positive.

Results: Regarding RV detection, both ICG and conventional RT-PCR detected the presence of RV in 34.3% (72/210) samples, whereas the VIASURE Rotavirus Real Time PCR assay detected 42.4% (89/210) positive specimens. The RIDAGENE Viral Stool Panel II

real-time RT-PCR detected 39% (82/210) RV-positive samples. NoV GI and GII were detected by conventional RT-PCR in 11.6% (21/181) and in 24.8% (45/181) samples, respectively. The analysis of the same specimens by the VIASURE real-time assays yielded 11.6% (21/181) NoV GI-positive and 27% (49/181) NoV GII-positive samples, whereas the RIDAGENE tests yielded 13.2% (24/181) NoV GI-positive and 27% (49/181) NoV GII-positive results. The VIASURE Norovirus GI and GII assays detected the following NoV genotypes: GI.1, GI.2, GI.4, GI.9; GII.1, GII.4 (five variants), GII.7, GII.17, and GII.21.

Conclusions: The sensitivity of the VIASURE Rotavirus Real Time PCR assay increases 1.23 times those of the ICG assay and the end-point RT-PCR. Rotavirus was detected by both evaluated real-time assays with a total agreement rate of 94.8%. For norovirus GI and GII detection, the total agreement rate between VIASURE and RIDAGENE real-time assays was 96.1% and 98.9%, respectively. The specificity of the assays is pending on the sequence analysis of the mismatched positive samples.

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Abstract no: 25
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Epidemiology of Astrovirus infection in infants in Tehran, Iran

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Background: Human astroviruses (HAstVs) are positive-sense single-stranded RNA viruses that were no enveloped particles with a distinctive star-shaped surface structure that have been were discovered in 1975 by electron microscopy in stool samples [1,2]. Human HAstVs classified into the genera Mamastrovirus and Astrovirus and have emerged as another common cause of non-bacterial acute gastroenteritis [3]. Limited data exist on the epidemiology and genetic diversity of HAstVs in Iran. This study assessed the role of human Astrovirus (HAstV) at the 120 gastroenteritis samples to determine the prevalence, time distribution, and medical significance of human Astrovirus infection among children 5 years old in Tehran, Iran.

Materials and methods: Stool samples were collected from Children's Medical Centers in Tehran, Iran from May 2013 to May 2014. HAstV was detected using RT-PCR and positive samples were subsequently tested for other common viral as well as Rotavirus and Norovirus [4].

Results: The overall incidence of Astrovirus was found to be 6.6%. Mixed infections with other viral enteric agents were detected in 2.5% of all Astrovirus-positive samples. During the 1-year period, the highest Astrovirus incidence was reported in the winter months, although infections also occurred in spring.

Conclusion: From our data for each age group, we observed that HAstV affected mostly children younger than 1 years of age, while HAstV had a greater impact in Male children. Our study provides an epidemiological overview of HAstVs associated with acute diarrhea in Iran.

Reference

[1] B.B. Munnink, L.V. Hoek, Viruses causing gastroenteritis: the known, the new and those beyond, Viruses 8 (2) (2016) 42.
[2] P.C. Woo, S.K. Lau, J.L. Teng, A.K. Tsang, S. Joseph, J. Xie, S. Jose, R.Y. Fan, U. Wernery, K.Y. Yuen, A novel astrovirus from dromedaries in the Middle East, J. Gen. Virol. 96 (9) (2015) 2697–2707.
[3] D.K. Mitchell, D.O. Matson, X. Jiang, T. Berke, S.S. Monroe, M.J. Carter, M.M. Willcocks, L.K. Pickering, Molecular epidemiology of childhood astrovirus infection in child care centers, J. Infect. Dis. 180 (2) (1999) 514–517.
[4] F. Sidoti, M. Rittà, C. Costa, R. Cavallo, Diagnosis of viral gastroenteritis: limits and potential of currently available procedures, J. Infect. Dev. Countries 9 (06) (2015) 551–561.

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Abstract no: 29
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Surveillance of Noroviruses GII.4 variants, GII.3 and GII.17 in acute gastroenteritis patients in Shenzhen, China from 2012 to 2015

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Background: Norovirus (NoV) is a leading cause of acute gastroenteritis (AGE) in individuals of all ages. Shenzhen, an immigrant metropolis connecting mainland China and Hong Kong, has a high population density and mobility, which make Shenzhen an AGE-prone area allowing for centralized surveillance of NoV-associated activities. The goals of this study were to determine the etiology of NoV-associated AGE and to figure out the epidemiological characteristics of NoV epidemic strains (GII.4 Den Haag_2006b, GII.4 Sydney_2012, GII.3 and GII.17) in Shenzhen, China.

Methods and specimens: From 2012 to 2015, a total of 4432 stool specimens were collected with AGE patients' data. There were 18 different NoV serotypes identified by real time RT-PCR and one step RT-PCR with N/S primers GI-SKF/GI-SKR and COG 2F/G2-SKR, including GII.4 Den Haag_2006b, GII.4 Sydney_2012, GII.4_can not assign, GII.2, GII.3, GII.6, GII.7, GII.12, GII.13, GII.17, GII.21, GI.1-GI.6 and GI.8. Results, from 2012 to 2015, GII.4 Sydney_2012 accounted for the majority proportion of AGE (29.41–75.83%), followed by GII.3 (2.61%-10.00%). GII.4 Den Haag_2006b (49.57%) was the predominant serotype in 2012. As the novel pathogen in Shenzhen, GII.17 (10.43–21.74%) became as a common causative agent of AGE in the winter season of 2014–2015. Statistical analysis indicated that the median (QR) age of AGE patients infected with GII.4 Sydney_2012 (21.50 [31.75]) was the highest compared to GII.4 Den Haag_2006b (1 [3.83]) and GII.3 (0.58 [0.58]) ($P < 0.001$) in 2012; the median (QR) age (1.00 [32.25]) of GII.4 Sydney_2012-infected AGE patients was higher than that (0.58 [2.17]) of GII.3-infected AGE patients in 2013 ($P < 0.05$); the median (QR) age (30.00 [26.25]) of GII.17-infected AGE patients was the highest compared to that of GII.4 Sydney_2012 (1 [12.33]) and GII.3 (0.67 [10.58]) ($P < 0.001$) in 2014, which was similar to that in 2015. The male/female ratio was different among these four prevalent NoV serotypes in 2012, 2014 and 2015. Phylogenetic analysis based on N/S gene revealed that NoV strains in Shenzhen were closely homological to that in the neighbour cities and countries.

Conclusion: The diversity of NoV has been surveilled that GII.4 variants, GII.3 and GII.17 were the prevalent strains in Shenzhen, China, from 2012 to 2015. Continued monitoring and comprehensive analyses of NoV is necessary for a better understanding of their evolution, epidemiology, prevention and control measures.

Keywords: Norovirus, Acute gastroenteritis, GII.4 variants, GII.3, GII.17.

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Abstract no: 293
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Investigation of the role of gastroenteritis causing viruses in the asymptomatic neonatal digestive tract

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Background: Viral gastroenteritis is a significant cause of morbidity and indeed mortality across the world. The World Health Organization (WHO) estimates that every year there are 1.7 billion cases of diarrhoeal disease, with the suggestion is that neonates and children under 5 years old are particularly prone. However little is known about the role of viruses in asymptomatic infants. This study sets out to identify whether certain viruses associated with diarrhoeal disease are present in asymptomatic neonates.

Methods: Faecal samples from 245 symptomatic neonates, 80 symptomatic 0–5 year olds, and 113 asymptomatic neonates were collected from patients attending the Royal Infirmary of Edinburgh and analysed for the presence of Enterovirus, Parechovirus, Astrovirus, Sapovirus, Norovirus, Rotavirus, Adenovirus and Human Bocavirus by Real Time PCR. Ethical approval was obtained to collect faecal samples from asymptomatic neonates. All samples were analysed with no patient identifiable data. Results were entered into a database and analysed. A comparison of these groups was then performed.

Results: 19.2% and 27.5% of symptomatic neonates and 0–5 year olds, respectively, tested positive for at least one of the viruses. Enterovirus was the most common of the viruses, whereas parechovirus, astrovirus, and sapovirus were rarely found. No strong gender bias, nor age-bias was identified. Enterovirus was found in 18.6% of asymptomatic neonates. Furthermore, this study showed the presence of Adenovirus DNA in 27% of asymptomatic children, which was higher than the prevalence of 11% seen amongst symptomatic children. No Norovirus, Rotavirus or Human Bocavirus infections were detected in the asymptomatic cohort, but were detected in 2.8%, 4% and 4.62% of symptomatic patients respectively. Moreover, levels of viruses detected in patients older than 3 months of age were statistically higher than in their younger counterparts ($p = 0.001$).

Conclusions: This study concludes that Adenovirus is the most prevalent virus in the asymptomatic neonate population. Enterovirus is the most prevalent virus found in both symptomatic and asymptomatic populations of children within NHS Lothian of less than five years of age. Due to the appearance of enterovirus in both groups it cannot conclusively be linked with a disease-causing phenotype, and highlights that these viruses may form part of the natural microbiome in young children.

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Abstract no: 315
Presentation at ESCV 2016: Poster 89

A paediatrics case with unusual presentation of adenovirus enteritis in immunocompetent host

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Adenovirus is the second most common cause of gastroenteritis in children in the UK after rotavirus. Infection is usually mild and self-limiting in the immunocompetent host. Complications are uncommon, and the management is mainly supportive therapy.

We present a case of 7 years old boy, otherwise fit and healthy, presented with on going problems with pale stools, nausea, abdominal pain and lethargy. The course of his symptoms was protracted and affected his nutritional status. In addition, he had a history of multiple food intolerances and dysphagia. He had been kept on gluten-free diet for many years, which improved his diarrhoea.

He was investigated for possible coeliac disease, and was reintroduced to gluten for 3 months to allow for an accurate biopsy results, but has found recurrence of diarrhoea and worsening of symptoms once gluten was reintroduced. Upper GI endoscopy was performed and mucosal biopsies were taken to rule out coeliac disease. Examination of the biopsies from the duodenal lining was not in keeping with coeliac disease. There was however, evidence of what is commonly seen with current infections. Electron microscopy was performed on formalin-fixed, paraffin-embedded duodenal biopsies. Intra-nuclear inclusions of viral particles were seen. Particles were hexagonal in shape with an average diameter of 68 nm and arranged in regular array formations. PCR analysis was performed on 4 curls from the duodenal biopsy material, and the 4 samples were all positive to adenovirus DNA. In addition, each of the 4 biopsies was PCR negative to astrovirus, rotavirus, sapovirus, enterovirus, parechovirus, norovirus, cytomegalovirus and Epstein-Barr virus.

The current case represents an unusual and protracted course of adenovirus enteritis in immunocompetent host. Adenovirus infection in this child was associated with prolonged course, malnutrition and lactose intolerance. It is not clear in this patient whether adenovirus infection is the direct cause of his symptoms, or it is an associating factor to another undiagnosed clinical condition. Secondary lactose intolerance has been reported as a rare complication of acute gastroenteritis in children, however little is known in literature about the mechanism, rates and correlation with cases of adenovirus enteritis. Further investigation in this field is required in order to shed more light on the possible mechanisms, and prevent missed opportunities of clinical diagnosis.

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Abstract no: 100
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Hepatitis A virus epidemiology in Turkey as childhood vaccination begins: Seroprevalence and endemicity by region

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Introduction and aim: Hepatitis A virus (HAV) is usually acquired through contact with an infected person or through ingestion of contaminated water or food, and it is one of the major causes of acute viral hepatitis globally [1]. The incidence of the disease varies with access to clean drinking water and other indicators of socioeconomic status [2,3]. Turkey introduced hepatitis A virus (HAV) into its routine childhood immunization program in 2012. Over time, this will change the age-seroprevalence profile of the country. This study provides a baseline evaluation of hepatitis A endemicity rates by province prior to the initiation of the vaccination program.

Methods: A systematic review of all hepatitis A serosurveys that collected data between 2000 and 2015 and published their results in English and Turkish was conducted. The systematic review was conducted in accordance with the PRISMA guidelines [4].

Results: In total, 51 studies from 23 of the 81 provinces in Turkey were identified, although for most provinces the quality of the data was poor. Adult prevalence rates were high and similar across the country. Child prevalence rates were lower in the western and central regions than in the eastern region. The age at midpoint of population immunity was in the teenage years for the west and central regions (intermediate endemicity), while the midpoint was in children less than 10 years old in the east (high endemicity). However, there was significant heterogeneity by province.

Discussion and conclusions: Provinces with a more urban population tended to have intermediate endemicity and provinces with a more rural population tended to have high endemicity. This prediction does not suit to the provinces where has migration areas and undeveloped neighborhood of the provinces cause increase in endemicity [5].

Turkey's current universal childhood vaccination recommendations are appropriate one based on the current endemicity status. The incidence rate will likely further decrease as a function of both the vaccination program and ongoing infrastructural development.

Keywords: Hepatitis A virus, Endemicity, Seroprevalence, Vaccination, Socioeconomic development.

Reference

[1] K.H. Jacobsen, S.T. Wiersma, Hepatitis A virus seroprevalence by age and world region, 1990 and 2005, Vaccine 28 (2010) 6653–6657.
[2] K.H. Jacobsen, J.S. Koopman, Declining hepatitis A seroprevalence: a global review and analysis, Epidemiol. Infect. 132 (2004) 1005–1022.
[3] K.H. Jacobsen, J.S. Koopman, The effects of socioeconomic development on worldwide hepatitis A virus seroprevalence patterns, Int. J. Epidemiol. 34 (2005) 600–609.
[4] D. Moher, L. Shamseer, M. Clarke, D. Ghersi, A. Liberati, M. Petticrew, et al., Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement, Syst. Rev. 4 (2015) 1.
[5] Z. Kurugol, A. Aslan, E. Turkoglu, G. Koturoglu, Changing epidemiology of hepatitis A infection in Izmir, Turkey, Vaccine 29 (2011) 6259–6261.

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Evaluation of anti-HCV Line immunoassay indeterminant results

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Introduction: Chronic hepatitis caused by hepatitis C virus infection is one of the leading causes of liver cirrhosis and hepatocellular carcinoma globally. Testing for HCV infection begins with the detection of antibodies to recombinant or synthetic HCV proteins using enzyme immunoassays (EIA). Because of the false positive results especially in low prevalence settings, positive anti-HCV EIA results are usually confirmed by recombinant immunoblot tests and Line immunoassays (LIA). The current clinical practice after identifying a positive anti-HCV result is to measure HCV RNA to assess whether viremia is present. The main problem related to LIA testing concerns the indeterminate results. The aim of this study is to determine the frequency of LIA indeterminant results in our routine practice and to evaluate the characteristics of these samples.

Materials and methods: A total number of 245 anti-HCV Line Immunoassay (Innogenetics Ghent, Belgium) results previously tested in Ege University Hospital, Department of Medical Microbiology Virology Laboratory between January 2013-August 2015 were reviewed. All the samples were positive with Architect Anti-HCV assay (Abbott Laboratories, IL, USA) run on the i2000SR analyser.

Results: Between January 2013 and August 2015 a total number of 81,948 samples were sent to Ege University Medical Microbiology Virology Laboratory for anti-HCV EIA testing. Of these samples 2576 (%3.14) were reactive, and 79,372 (%96.86) were nonreactive. During this period 245 samples mostly with low signal to cutoff ratios were tested by LIA. Of the 245 samples, 49 were positive (20%), 155 were negative (63.3%) and 41 were indeterminant (16.7%) by line immunoassay testing. Of the patients with indeterminant LIA results, 22 were female and 19 male, ages ranging 1-84 (mean 41.1 ± 16.1). The distribution of Architect reactivity ratios expressed as s/co of these samples were between 1.01 and 8.49 (mean s/co 2.3) and 37 samples had s/co < 5.0. 39 samples had HCV RNA results and HCV RNA positivity was recorded in only one patient with s/co ratio 6.3 and NS3 band reactivity. Of the samples with indeterminant results 29 samples presented reactivity to NS3 antigen, 7 samples to C1 antigen, 3 samples to C2 antigen, one to E2 and one to NS4 antigen.

Conclusion: Overall there are 41 indeterminate samples out of 245 samples tested. Most of the samples are below the s/co index value <5.0. All the indeterminate samples were PCR negative except one patient. Some possible causes for indeterminant results are seroconversion phase during which EIA is already positive and seroreversion in patients who spontaneously eliminate HCV. In these individuals antibodies against some antigenic fractions have already turned negative for LIA but they are sufficient to cause an EIA positive result and other factors related to kit performance or to patient immunoresponse variability may be involved. The other possibility that must be considered is false positive EIA result.

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Memory T cells specific for HBV enumerated by a peptide-based cultured enzyme-linked immunospot assay in healthy HBV-vaccinated subjects

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Background: Hepatitis B vaccine is the most effective strategy to control hepatitis B virus (HBV) infection and disease. It is considered that an anti-HBs (antibodies against HBV surface antigen) titer >10 mIU/ml, measured shortly after a complete vaccination schedule, provides protection against infection. Approximately 4–10% of healthy individuals fail to respond to three doses of vaccine. Additionally, an estimated 13–60% of initial responders to HBV vaccine (>10 mIU/ml after three doses) may lose anti-HBs after several years post-vaccination. The aim of this study was to evaluate the long-term HBV-specific memory T-cell response in healthy vaccinated subjects.

Materials and methods: We quantified HBV-specific expandable memory T cells by using a peptide-based cultured IFN-gamma enzyme-linked immunospot following 10 days stimulation (cultured ELISPOT). Response to an overlapping peptide pool (15-mers overlapping by 11 amino acids) representing the complete L (large) HBV envelope polypeptide was evaluated in 46 healthy subjects (mean age of 36.24 years, standard deviation (SD) = 10.48; 12 males and 34 females). Forty-one subjects (89.1%) were vaccinated for HBV about 15–20 years earlier. Plasma samples were tested for anti-HBs.

Results: We observed that vaccinated subjects had significantly higher HBV-specific T-cellular response than unvaccinated ($p = 0.0002$). HBV-specific memory T-cell response quantified by cultured ELISPOT was mainly mediated by $CD4^+$ T cells. No concordance was found between cultured ELISPOT and anti-HBs data in vaccinated subjects. Thirty-one (76%) vaccinated subjects were responders (anti-HBs >10 mIU/ml), while 10 (34%) were non-responders (anti-HBs <10 mIU/mL). Nineteen (46%) vaccinated subjects were considered to be responders in the HBV-specific cultured ELISPOT assay. Twenty-two (54%) vaccinated subjects were considered non-responders in the HBV-specific cultured ELISPOT; five of them (23%) were also humoral non-responders. About 12% of healthy HBV-vaccinated subjects were both humoral and cellular non-responders. Thus, these subjects may be at risk for HBV infection and disease, especially health care workers.

Conclusion: In conclusion, the evaluation of HBV-specific T-cell response by cultured ELISPOT may represent a new tool to monitor memory immunity to HBV vaccine in immunocompromised patients, such as hemodialyzed patients or patients who underwent solid organ transplantation, that are at high risk for infection.

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Impact of the genotyping method on the distribution of hepatitis C virus subtypes of genotype 1

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Background: At least 6 major hepatitis C virus (HCV) genotypes (1–6) and multiple subtypes (e.g. a, b, c) have been identified, based on the sequence differences. Distribution of certain genotypes in the patient population is highly dependent on geographical region but also differs by gender, ethnicity, age and mode of transmission. Differences in the patient population regarding subtypes of HCV genotype 1 have also been reported: e.g. patients older than 65 years are more likely and injecting drug users are less likely to harbour subtype 1b than 1a. In the present study we investigated the potential impact of the change in the laboratory method used for HCV genotyping on the subtype distribution of HCV genotype 1.

Materials and methods: Samples submitted for HCV genotyping at the Ghent University Hospital from January 2002 until December 2014 were included in the current study, based on the retrieval of results through the laboratory information system. HCV genotyping results during this period of 12 years were obtained by the reverse hybridization based Versant HCV Genotype Assay but 2 different versions of the same assay were used: from January 2002 till March 2007 Versant HCV Genotype 1.0 was used and from April 2007 till December 2014 Versant HCV Genotype 2.0. The improvement of the second generation assay, including the core region probes, lies mainly in the distinction between subtype a and subtype b of genotype 1. Therefore, we focused on the possible changes in distribution for subtypes of genotype 1.

Results: From January 2002 till December 2014, 1631 serum samples were determined positive for HCV genotype 1. For 853 genotype 1 positive samples analyzed with Versant HCV Genotype 1.0 from January 2002 till March 2007, 23 (2.7%) samples were attributed to subtype 1a; 716 (83.9%) samples to 1b and 114 (13.4%) genotype 1 positive samples could not be subtyped further. For 778 genotype 1 positive samples analyzed with Versant HCV Genotype 2.0 from April 2007 till December 2014, 271 (34.8%) samples were attributed to subtype 1a; 493 (63.4%) samples to 1b and only 14 (1.8%) genotype 1 positive samples could not be subtyped further with this advanced version of the same assay.

Conclusions: In the current study we demonstrate that the switch of the version of the reverse hybridization based Versant HCV Genotype Assay had a major impact on the distribution of subtypes of HCV genotype 1 bringing subtype 1a from less than 3% of genotype 1 samples in the first version – based only on the analysis on 5′-UTR – to more than one third of the genotype 1 samples, as analyzed by the advanced version of the same assay where core-region analysis was added. The number of non-subtyped genotype 1 samples has been reduced by version switch from more than 13% to less than 2%. Evaluation of the potential impact of other factors that can influence change in distribution of subtypes of HCV genotype 1 as age of the patients and the mode of transmission still has to be performed. However, the current use of the improved ver-

sion of genotyping is most likely to be largely responsible for the mentioned change in distribution of subtypes.

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Abstract no: 160
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Hepatitis E virus seroprevalence in East- and West Flanders, Belgium: Comparison between 2011 and 2016

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Background: Hepatitis E virus (HEV) infection is increasingly recognized as a cause of hepatitis in developed countries. There are wide variation in HEV seroprevalence between countries and regions. Moreover, some authors report changing seroprevalence numbers over time. Differences in performance characteristics between serological assays used could explain some of these findings. However, significantly different seroprevalence figures are also reported using the same assay. The objective of this study was to evaluate the current HEV seroprevalence in East- and West Flanders, Belgium and to assess the evolution of the seroprevalence over time.

Materials and methods: In the setting of the Ghent University Hospital, samples of 200 patients (100 between June 29th and July 2nd 2011 and 100 between May 9th and May 12th 2016) without clear evidence of hepatitis nor gastroenterological problems were randomly selected for anti-HEV IgG testing (Wantai Biological Pharmacy, Beijing, China). The median age of the selected individuals was 39 (range 17–82 years) and 37 (range 18–82 years) years old in 2011 and 2016 respectively. In both groups the male/female ratio was 1.

Results: 15/100 samples collected in 2011 and 15/100 samples collected in 2016 tested positive for anti-HEV IgG. Both study groups (2011 and 2016) showed an increasing seroprevalence with age. In 2011, we found a similar anti-HEV IgG seroprevalence for men (16%; 8/50 patients) and women (14%; 7/50 patients). In the 2016 subgroup, the HEV seroprevalence seems to differ according to sex. A higher seroprevalence (24%; 12/50 patients) was found for men in comparison with women (6%; 3/50 patients). This difference was statistically significant (Fisher's exact test $p = 0.22$).

Conclusion: East- and West Flanders in Belgium, with HEV seroprevalence of 15%, can be classified as region with moderate seroprevalence. There seems to be no increasing trend over the last 5 years. The observed predominance of HEV IgG positivity in men over women in 2016 has to be further explored.

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Abstract no: 166
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Lyophilising have to create a stable product, is it possible?

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Picornaviruses are notoriously difficult to lyophilise without compromising viral integrity, however the value of producing stable hepatitis A viral reference materials in this format is paramount to assure assay sensitivity for the detection of both contamination of blood derived medicinal products and patient diagnosis.

NAT assays are readily available for HAV RNA detection, however in-house assays are predominant and typically have variations in sensitivity and specificity. Without the availability of suitable reference materials it is then impossible to compare results between laboratories or different assays. A WHO International Standard for Hepatitis A Virus RNA NAT assays has been available since 2000 and has helped considerably in the harmonisation of these assays. However, whilst establishing a replacement HAV IS, the stability of lyophilised picornavirus came into question. Accelerated thermal degradation samples of two replacement batches suggested that there was a loss of potency of up to 1 $\log_{10}$ upon storage of lyophilised material at +4 °C over 5 years, with a second batch showing such losses after only 10.5 months, this was in comparison to the corresponding loss of potency for 1st HAV IS which was observed to be <0.1 $\log_{10}$.

Stability and homogeneity over time are critical attributes of a reference material. Stability is evaluated to provide an estimate of the length of time for which the reference standard will remain suitable for its intended purpose under its defined storage conditions.

We have undertaken a pilot study to investigate suitable formulations for minimizing loss in potency of HAV upon freeze drying and degradation in storage at elevated temperatures using a range of NAT assays prior to the development of the replacement HAV RNA IS this comprised for formulations tested by three laboratories using different assays.

In the pilot study, we collaborated with the Paul Erlich institute and Altona Diagnostics to analyse HAV freeze dried formulations stored at elevated temperatures in accelerated degradation studies. The analysis indicated the formulation with Trehalose and Hepes buffer, reduced initial loss in potency upon freeze drying and subsequent storage at elevated temperatures using a number of different NAT assays. The findings from this study guided the formulation for the replacement standard, which will be assessed in direct comparison to a standard formulated in plasma only over a long termin a multicenter collaborative study, involving a broader range of NAT-based assays. Each participant will be asked to evaluate dilution series of the candidate IS's for HAV alongside a panel of clinical samples.

We shall present the results of this second study to establish whether there is improvement in the stability for HAV reference materials using the new formulation. Two candidate formulations one with plasma only the other with excipients, freeze dried and liquid bulk samples will be compared using a range of assays to assess initial loss of potency. The implications of these data and suitability of the IS to improve the quality and comparability of NAT based assays for HAV will be discussed.

Keywords: HAV International Standard, Freeze drying, Stability.

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Abstract no: 177
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Characterization of NS3 region and frequency of resistance mutations against Simeprevir of hepatitis C virus genotype 1a and 1b in Portuguese infected patients

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Hepatitis C virus (HCV) is a significant public health problem and the leading cause of liver transplantation and hepatocellular carcinoma. Globally, approximately 180 million people are infected with HCV. There are seven genotypes of HCV, with a variety of subtypes. The Portuguese prevalence of HCV infection is about 1.0%, being the genotype 1 the most prevalent and one of the most difficult to treat with the previous therapies. However, the face of HCV therapy has changed dramatically since 2011 with all-oral regimens that have been emerging, characterized by high genetic barrier to viral resistance and low side effects. A new hope to cure HCV infected patients have arisen. Actually, in Portugal the first line treatment is Sofosbuvir plus Ledipasvir and in fact we have around 95% of sustained viral response from the treated patients. Those who still do not cure infection are proposed to Sofosbuvir plus Simeprevir, being the last one ineffective if in the genome of the virus are present some mutation such as Q80K, S122R/A/G, R155K/QD168E/V/H/A and I/V170T. Thus, the characterization of NS3 mutations has a highly impact on the prediction of the success of this direct antiviral agent. Q80K is observed predominantly in HCV genotype 1a as a naturally occurring polymorphism and seldom in other HCV genotypes. It ranges from 5% to 47%, depending on geographic region.

The objective of this study was to characterize HCV NS3 of Portuguese patients still naive to Simeprevir, once less is known in our population about the occurrence of natural mutations to this drug in that viral genomic region.

HCV NS3 region of 112 patients infected with 1a (89.3%) and 1b (10.7%) viral genotypes was sequenced with an in house sequencing method described previously by Harringan et al. and the analysis of NS3 amino acids 1–181 was performed using geno2pheno HCV.

The prevalence of known pre-existing resistance mutation Q80K was 3.36% in the whole sample. However, it was not found in genotype 1b and this turned the prevalence of Q80K in genotype 1a around 3,0%. Q80R resistance mutation was found in one sample of genotype 1a (1%) as other pre-existing polymorphisms at that same position, Q80H (2%), Q80L (1%) which are not associated to resistance but may act as intermediate mutations to Q80K. Additionally, it was found R155K (1%) and R155G (1%) in genotype 1a, both conferring resistance to Simeprevir. Notably, in genotype 1a there was also a higher occurrence of the substitutions T40A (62%), N174S and N174G (71%), S91A (98%) and L153I (100%) which have rarely been reported in the literature. T40A and S91A involve changes in the amino acid's electrical charge and position 91 is in close proximity to the residues in the catalytic triad. L153I does not lead to this kind of amino acid's changes, but based on its high frequency in this population, it may be a regional genetic polymorphism and surprisingly, there were found 71% of samples simultaneously presenting L153I, S91A and N174S/G which may suggest an association of substitutions.

In conclusion, there were not just detected 5% of preexisting mutations that cause resistance to Simeprevir, but also some polymorphisms that should be further investigated not only by their notably association, but also because the changes they promote near the catalytic center of the enzyme, that can be a cause of some still unknown protease inhibitor treatment failures too.

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Abstract no: 178
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Hepatitis B virus infection and reactivation in two patients with anti-HBs following etanercept therapy and alemtuzumab and rituximab therapies: Case presentation

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HBV reactivation is a well known phenomenon following cytotoxic or other immunosuppressive anticancer therapy. Biologic therapies with monoclonal antibodies lead to hepatitis B virus (HBV) reactivation. It is aimed to present and discuss two hepatitis B cases following therapies with monoclonal antibodies to emphasize HBV assessment in patients receiving biologic therapy.

Materials and methods: HBV serological test results are evaluated with the previous markers of the cases. HBsAg, anti-HBc, IgM anti-HBc and anti-HBs are tested with Architect system (Abbott Diagnostics, Germany). HBV viral load is tested with Abbott *m2000sp* and *m2000rt* (Abbott Molecular Diagnostics, USA). Cases with markers of HBV infection who were HBsAg negative and anti-HBs positive are evaluated retrospectively and with clinical history.

Case 1: A man with rheumatoid arthritis had HBV DNA 7.14 $\log_{10}$IU/ml, HBsAg positive, anti-HBc positive, anti-HBe positive, anti-HBs negative, IgM anti-HBc negative. Liver function tests were elevated (>2 N). He received lamivudin and seroconverted to anti-HBs positivity, HBsAg negative, but HBV DNA was still 1.34 $\log_{10}$IU/ml. Liver function tests turned to normal values. He was HBsAg negative, anti-HBc positive and anti-HBs 13 mIU/ml six months ago. He had received etanercept therapy six months ago. It was found that he had chronic HBV infection four years ago, but seroconverted in three years. He had no HBV DNA data beforehand, but found to be positive as 2.18 $\log_{10}$IU/ml when tested in archived samples.

Case 2: 64 years old male with cirrhosis was found to be HBsAg and HBV DNA positive (8.44 $\log_{10}$IU/ml), anti-HBc positive, anti-HBs negative. He was HBsAg negative, anti-HBc positive, anti-HBs 232 mIU/ml before. He had chronic lymphocytic leukaemia-small lymphocytic lymphoma and received alemtuzumab four years ago. He had graft versus host disease after allogeneic bone marrow transplantation and received rituximab three years ago. At that time he was HBsAg and anti-HBc negative, anti-HBs 159 mIU/ml.

Conclusion: Hepatitis may be observed also in subjects with anti-HBs positivity or with occult HBV infection with markers of previous exposure to HBV. HBV assessment in patients receiving biologic therapy (anti-TNF-alpha, anti-CD20, anti-CD52) is important and should be always remembered. HBsAg, anti-HBc, anti-HBs, ALT and AST should be tested. Quantitative HBV DNA test should be applied if HBsAg and/or anti-HBc are positive. Antiviral ther-

apy must be considered according to HBV DNA and HBV risk with closely monitoring.

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Abstract no: 191
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The distribution of hepatitis C virus genotypes of patients with chronic hepatitis C infection in Eskisehir Region of Turkey

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Chronic hepatitis C is a serious disease than can result in long-term health problems. At least 6 major HCV genotypes and more than 100 subtypes were determined. It is known that different genotypes in HCV infections account for differences in disease courses and treatment responses. In our study, it is aimed to determine HCV genotype distribution to suggest treatment responses of patients with chronic hepatitis C infection.

In this study, anti-HCV, HCV RNA viral loads and HCV genotypes of 203 patients followed-up in Eskisehir Osmangazi University Medical Faculty between 2009 and 2014 were investigated. Anti-HCV was tested by microparticle ELISA (Abbott AxSYM System HCV 3.0). HCV-RNA viral loads were determined by Artus HCV RG PCR kit (Qiagen, Germany) on Rotor-Gene 6000 (Corbett Research) instrument after extraction by Biorobot M48 system (Qiagen, Germany) between 2009 and 2011, and by Cobas TaqMan 48 (Roche, Germany) system after extraction by Cobas AmpliPrep (Roche) between 2011 and 2014 by Real Time PCR. HCV genotyping of HCV RNA positive patients was performed by HCV genotype Pyrosequencing test (Qiagen, Germany).

Eighty-seven (42.86%) of 203 patients were male and 116 (57.14%) were female. The average age of the patients was as 54.97 and the age range was 14–77. The distribution of HCV genotypes was as following: in 151 (74.4%) patients genotype 1; in 3 (1.4%) genotype 2; in 4 (1.9%) genotype 3; in 4 (1.9%) genotype 4. In 151 patients who were positive for genotype 1, genotype 1b was positive in 36 (17.7%) and 3–8.35 $\times$ 10^7. In 191 (94.0%) patients anti HCV was positive and in 12 (6.0%) anti HCV was negative.

The most common HCV genotype in chronic hepatitis C patients followed up in Eskisehir region was genotype 1, and the most common subtype in this group was genotype 1b. Treatment protocols should be reevaluated by taking into consideration that sustained viral response in these patients might be weak. In Turkey, approximately 90% of HCV infections are by type 1 (most are type 1b), and type 2, 3, and 4 HCV infections are seen.

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Abstract no: 194
Presentation at ESCV 2016: Poster 99

Change in the prevalence of hepatitis E virus in the last 15 years, Turkey

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Objective: Hepatitis A, B and E infections are community health problems in developing countries and the most common acute viral hepatitis in children. Hepatitis E virus (HEV) causes epidemics in developing countries. Turkey represents a bridge between HEV endemic and non-endemic areas, and HEV may cause epidemics in Turkey. The epidemiology of HEV infections are best defined by measuring humoral antibodies in children. For this reason the purpose of this study was to detect the change in the prevalence of HEV antibody by systematic reviews in published medical literature from 2000 to 2015 years in Turkey.

Materials and methods: The study was planned and conducted in accordance with the declaration of PRISMA. To find the published series, two national databases (ULAKBIM and TURK MEDLINE) and one international database (PubMed) were investigated. Published manuscripts were evaluated according to the determined criteria for acceptance and rejection. For each study, anti-HEV IgG and anti-HEV IgM antibody rates were collected as a common unit.

Results: After screening according to the applied acceptance and rejection criteria, 13 studies published between 2000 and 2015 were included in the study for evaluating HEV antibodies seroprevalence. Anti-HEV IgM 4.15 $\pm$ 4.73 and anti-HEV IgG 4.24 $\pm$ 4.67 (mean $\pm$ SD) between 2000 and 2015. Anti-HEV IgG seroprevalence under the five years 2.56 $\pm$ 2.63, five to nine years 2.00 $\pm$ 2.47, 10–16 years 2.03 $\pm$ 2.73, respectively. It has been defined that the ratio of anti-HEV IgG changed between 0% and 17.3% in different studies done between the years 2000 and 2015 in our country.

Conclusion: In conclusion, we evaluated more than 5000 Turkish children HEV antibody prevalence more than 15 years period. Frequency of HEV infection varies greatly depending on geographic region, socioeconomic level, age and various risk factors. To take preventive measures to protect themselves from infection with HEV is important to know the prevalence of HEV.

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Abstract no: 211
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Hepatitis E virus subgenotypes 3i and 3f in wastewater of treatment plants of Portugal

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Introduction: Genotype 3 hepatitis E virus (HEV3) is widespread in industrialized countries and infection in humans is mostly the result of foodborne zoonotic transmission from swine, considered the most important animal reservoir for human disease [1]. Since HEV is excreted in human and animal stools, it has the potential to be introduced in aquatic environments through urban and agriculture runoff, sewage outfall and vessel wastewater discharge. The involvement of water in the transmission of HEV3 infection is uncertain but this hypothesis has been drawing increasing attention from the scientific community [2]. Herein, we describe the first detection and molecular characterization of HEV3 isolates retrieved from Wastewater Treatment Plants (WWTP) of Portugal.

Materials and methods: A total of 60 influent and effluent wastewater samples from 15 WWTP, located in the 5 Portuguese regions, namely North, Centre, Lisboa and Vale do Tejo, Alentejo and Algarve were studied. From each WWTP, time proportional 24-h composite influent ($N = 1$) and effluent ($N = 1$) samples were collected in September and in December 2013. Viral concentration was performed according to the previously described method [3]. A broad RT-qPCR probe assay targeting the open reading frame (ORF) 2 region of the HEV was performed [4]. Samples positive by RT-qPCR were submitted to a nested broad-spectrum RT-PCR with amplification within the ORF1 region of HEV genome [5].

Results: HEV RNA was detected in 2 of the 60 wastewater samples tested. They were both influent wastewater samples collected in December, one from a WWTP located in the North, and the other located in the Centre of Portugal. Phylogenetic analysis showed that both isolates belonged to genotype 3 but clustering in different subgenotypes, namely 3i and 3f. These isolates presented only 79.2% nucleotide sequence homology, showing genetic heterogeneity among environmental isolates.

Discussion: Molecular diagnostic tools are known to be valuable for environmental surveillance, assisting in the assessment of the epidemiology of the circulating viral community in a given population [3]. As far as we know this is the first study investigating the presence of HEV in WWTPs of Portugal, and providing evidence of the circulation of different HEV3 strains in this environment.

Reference

[1] A. Berto, J.A. Backer, J.R. Mesquita, et al., Prevalence and transmission of hepatitis E virus in domestic swine populations in different European countries, BMC Res. Notes 25 (2012) 190.
[2] W.H. Van der Poel, Food and environmental routes of Hepatitis E virus transmission, Curr. Opin. Virol. 4 (2014) 91–96.
[3] A. Rafique, S. Jiang, Genetic diversity of human polyomavirus JCPyV in Southern California wastewater, J. Water Health 6 (2008) 533–538.
[4] K.J. Rolfe, M.D. Curran, N. Mangrolia, et al., First case of genotype 4 human hepatitis E virus infection acquired in India, J. Clin. Virol. 48 (2010) 58–61.
[5] R. Johne, A. Plenge-Bönig, M. Hess, et al., Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR, J. Gen. Virol. 91 (2010) 750–758.

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Abstract no: 225
Presentation at ESCV 2016: Poster 101

Seroprevalence of hepatitis B in children in Konya region of Turkey

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Background: Routine hepatitis B vaccination program of Ministry of Health Services has been performed since 1998 in Turkey. Since then Hepatitis B infection is gradually decreased as a result of vaccination. We aimed to determine the ratio of anti-HBs and HBsAg seropositivity in children who was born after implementation of hepatitis B vaccine.

Methods: Between 2013 January and 2016 January, children aged 0–18 years, who were admitted to Konya Public Health Laboratory, were included in this study. Venous blood samples were tested for Hepatitis B surface antigen (HBsAg) and Hepatitis B surface antibody (Anti-HBs) by enzyme-linked immunosorbent assay.

Results: The rates of HBsAg and Anti-HBs were found 0.85% and 75% respectively in child. HBsAg prevalence was 0.7% in children between 0 and 6 years; 0.9% between 6 and 12 years; 0.8% between 12 and 18 years old. Anti-HBs rate was 82.6% in children between 0 and 6 years; 70.3% between 6 and 12 years; 76.5 between 12 and 18 years old. Of HBsAg (+) children, 56% were females and 44% were males.

Conclusion: National immunization scheme caused a significant decrease in HBsAg positivity. The ratio of AntiHBs seropositivity is increasing in subjects born after implementation of hepatitis B vaccine, though not reached to targeted level yet.

Keywords: Anti-HBs, HBsAg, Seroprevalence, Children, Vaccination.

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Abstract no: 23
Presentation at ESCV 2016: Poster 102

Performance of the new Aptima HCV quant Dx assay in comparison to the Cobas TaqMan HCV2 assay for use with the high pure system in the detection and quantification of HCV RNA in plasma or serum

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Quantitating the level of hepatitis C virus (HCV) RNA is the standard of care for monitoring HCV-infected patients during treatment. The performances of commercially available assays differ for precision, limit of detection, and limit of quantitation. Here,

we compare the performance of the Hologic Aptima HCV quant Dx assay (Aptima) to the Roche Cobas TaqMan HCV test, version 2.0, using the high pure system (HPS/CTM), considered a reference assay since it has been used in trials defining clinical decision points in patient care. The assays' performance characteristics were assessed using HCV RNA reference panels and plasma/serum from chronically HCV-infected patients. The agreement between the assays for the 3 reference panels was good, with a difference in quantitation values of <0.5 log. High concordance was demonstrated between the assays for 245 clinical sample s (kappa = 0.80; 95% CI: 0.720–0.881); however, Aptima detected and/or quantitated 20 samples that HPS/CTM did not detect, while Aptima did not detect 1 sample that was quantitated by HPS/CTM. For the 165 samples quantitated by both assays, the values were highly correlated ($R = 0.98$; $P < 0.0001$). The linearity of quantitation from 1.4 to 6 log was excellent for both assays for all HCV genotypes tested (GT 1a, 1b, 2b and 3a) ($R^2 > 0.99$). The assays had similar total and intra-assay variability across all genotypes from 1000 to 25 IU/mL. Aptima had a greater analytical sensitivity, quantitating more than 50% of replicates at 25 IU/ml target. Aptima showed performance characteristics comparable to those of HPS/CTM and increased sensitivity, making it suitable for use as a clinical diagnostic tool on the fully automated Panther platform.

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Abstract no: 24
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Association of rs731236(Taq1)VDR TT genotype, chronic hepatitis B infection and serum vitamin D level

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Aim: The role of vitamin D in chronic hepatitis B (CHB) infection has attracted a lot attention, the aim of this study is to determine the association between rs731236(Taq1)VDR TT genotype, serum circulating 25-hydroxyvitamin D (25 (OH) D) among CHB patients.

Methods: This study enrolled one hundred and twenty nine CHB patients, one hundred and thirty thee healthy Jordanians as a control from Prince Hamzah and Al-Basheer Hospitals/Amman (2013-2015). 25 (OH) D level was determined by competitive immunoassay, Hepatitis B antigen (HBsAg) was determined by HBsAg ELISA and rs731236(Taq1)VDR TT genotype was determined by polymerase chain reaction.

Results: Mean level of serum 25(OH)D among CHB patients was significantly lower (11.2 ±15.1) compared to heathy control (19.3 ± 3.4). The percent of CHB patients with deficient, insufficient and optimal 25(OH)D was (55.8%, 28.6%, 15.5%) among CHB compared to (43.6%, 32.3 and 24.1%) among healthy control; respectively. Statistical difference was found in vitamin D level within rs731236(Taq1)VDR TT genotype among the CHB patients (10.6 ± 4.3 ng/mL) compared to its control (20.41 ± 3.3 ng/mL).

Conclusion: Vitamin D deficiency and rs731236(Taq1)VDR TT genotype are common among patients with CHB and is may be associated with adverse CHB clinical outcomes.

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Abstract no: 243
Presentation at ESCV 2016: Poster 104

Clinical evaluation of the Aptima HCV Quant Dx assay for hepatitis C virus RNA quantification on Panther system

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Background: The Hologic Aptima HCV Quant Dx assay (Aptima HCV) is a new real-time transcription-mediated amplification method for use in diagnosis and monitoring of HCV infection that runs on the fully automated Panther system in random access. Here we describe validation studies of the assay compared to the Cobas AmpliPrep/Cobas TaqMan HCV Quantitative Test, v2.0 (Roche HCV Quant) and Cobas AmpliPrep/Cobas TaqMan HCV Qualitative Test, v2.0 (Roche HCV Qual) in a clinical routine setting.

Methods: For method comparison 543 prospective (438) and retrospective (105) clinical plasma samples from HCV infected patients were tested side-by-side in Aptima HCV and Roche HCV to analyze concordance on qualitative results as well as correlation between quantitative results. Precision was tested using the PEI HCV-RNA Reference Standard (#3443/04, GT 1) diluted with negative plasma to 3 different target concentrations, 500, 100, and 25 IU/ml and tested in replicates of 21 each with the respective assays. Repeatability across different genotypes was demonstrated with and HCV genotype panel from the German NRC for HCV. Linearity was assessed by preparing serial dilutions of 4 well characterized clinical samples (GT 1a, GT 1b, GT 3, GT 6), with five dilution levels and target concentrations at 6 log IU/ml, 5 log IU/ml, 4 log IU/ml, 3 log IU/ml, 2 log IU/ml. Five replicates of each dilution level were tested side-by-side in the Aptima and Roche assays over 3 days, respectively. Accuracy was tested based on a current Instand HCV RNA EQA panel.

Results: With prospective clinical samples ($n = 223$), inter-assay agreement between Aptima HCV and Roche HCV Quant for qualitative results was high (92.4%) with Cohen's kappa statistic equal to 0.79. The inter-assay agreement between Aptima HCV and Roche HCV Qual for qualitative results was also high (95.8%) with Cohen's kappa statistic equal to 0.83. Of the 283 prospective and retrospective samples with quantitative results in both assays, Aptima HCV reported slightly higher values by an average of 0.2 $\log_{10}$ IU/mL, calculated according to Bland–Altman method, with Aptima yielding higher VLs in the upper VL range, and slightly lower VLs in the lower VL range (<5 log IU/ml). Concordance between assay results was high with a Pearson r concordance correlation coefficient of 0.98 ($P < 0.0001$). Both assays showed excellent linearity ($r > 0.98$) across different genotypes tested, with regression lines nearly parallel to the identity lines for Aptime HCV. Precision was 0.17 and 0.12 log SD or lower across the dilutions levels with the PEI standard and NRC HCV genotype panel, respectively, and superior to Roche HCV (<0.2 and <0.29 log SD, respectively). The Aptima HCV demonstrated excellent accuracy in the Instand HCV EQA panel.

Conclusion: The Aptima HCV demonstrated excellent precision, linearity, and accuracy in all genotypes tested. Good concordance was observed between Aptima HCV and Roche HCV when testing clinical samples from patients on therapy. This study has demonstrated that Aptima HCV meets the necessary requirements for HCV viral load monitoring and HCV diagnosis in a clinical routine setting.

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Abstract no: 251
Presentation at ESCV 2016: Poster 105

Reactivation of hepatitis B virus infection with pazopanib: Lessons for all in caring for co-morbid patients

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In an era of emerging immunosuppressive treatments for inflammatory and malignant conditions, hepatitis B virus reactivation (HBVr) is now a well described yet rare complication. From mild derangement of liver function to fulminant liver failure, clinicians must be aware of the risk. Pre-treatment screening can highlight those requiring prophylaxis or monitoring. We present a first case report of reactivation of resolved HBV (surface antibody [anti-HBs] negative, core-antibody [anti-HBc] positive), in a patient on pazopanib, a multi-tyrosine kinase inhibitor used in advanced renal cell carcinoma; a challenging case for all involved.

The patient was a Chinese male who began treatment in December 2015. He had undergone haemodialysis for over a year, screened according to local guidelines for hepatitis B surface antigen (HBsAg) 3 monthly and anti-HBs yearly (both undetected). Anti-HBc was not checked pre-pazopanib. A quarterly dialysis screen taken 8 weeks after starting pazopanib found low level HBsAg. This coincided with an assay issue causing false positive HBsAg results; significance of the result could have been missed but his ethnicity and dialysis status led to urgent confirmatory tests that showed low level HBV DNA. Testing of old stored samples showed resolved infection, with no evidence of HBVr on his dialysis screen 4 weeks pre-pazopanib, and no samples since to time the reactivation further. The patient had developed a transaminitis over the month prior to HBVr diagnosis, with pazopanib stopped by his oncologist. It is well known to increase transaminases; the patient was also taking pravastatin, further increasing the risk [1]. It was fortunate his routine dialysis HBV screen occurred at that time, since his liver function improved after stopping pazopanib and HBVr may have gone undetected.

HBVr in this patient had wider implications than just his health, as he was dialysing in an open ward until the diagnosis, and significant work required to follow up dialysis contacts. Fortunately the reactivated virus remained at low levels and no secondary HBV infections have occurred to date. Low level HBsAg is still detected although viral load is now undetectable and liver function recovered.

This case highlights the importance of HBV screening pre-immunosuppression, the need for clinicians to be aware of the risks of new therapies, and timely investigation and liaison between specialties in suspected adverse events. In Sheffield our oncologists do not routinely screen pre-treatment, also experienced elsewhere [2]. If past HBV was known, monthly screening would have been advised by local guidelines, and HBVr may have been detected earlier. Although pazopanib is not as high risk as some drugs, we are now aware that most immunosuppressive therapies have some risk of HBVr. With many new agents available, it is no longer just the prescribers that need to be aware of their complications, although focus on increasing screening by oncologists in particular, appears internationally to be the first step to protect such patients, and potentially others.

Reference

[1] C.F. Xu, Z. Xue, N. Bing, K.S. King, L.A. McCann, P.L. de Souza, et al., Concomitant use of pazopanib and simvastatin increases the risk of transaminase elevations in patients with cancer, Ann. Oncol. 23 (9) (2012) 2470–2471.
[2] S.A. Gonzalez, R.P. Perrillo, B. Hepatitis, Virus reactivation in the setting of cancer chemotherapy and other immunosuppressive, Drug Therapy Clin. Infect. Dis. 62 (Suppl. 4) (2016) S306–S313.

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Abstract no: 257
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Enhancing the screening of active hepatitis C virus (HCV) infection through molecular testing of dried-blood spots in a community-based counselling and testing centre in Barcelona

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Background: More than half of HCV-infected individuals are unaware of their disease, leading to transmission and liver disease progression. Promoting HCV screening is a global priority, but the conventional testing algorithm (serology followed by molecular confirmatory testing) does not facilitate the diagnosis of active infection, especially in hard-to-reach populations at risk. Thus, the use of alternative strategies is essential. HCV infection has emerged as a sexually-transmitted infection (STI) among HIV-infected men who have sex with men (MSM), but data in Spain on HIV-negative MSM as well as male sex workers (MSW) and transsexual women sex workers (TSW) is scarce.

Objectives: We aimed to set up a molecular assay able to detect the HCV-RNA in dried blood spots (DBS), and to assess its implementation and usefulness in comparison with antibody point-of-care testing in a community-based counselling and testing centre in Barcelona, in order to improve the early diagnosis of active HCV infection among MSM, MSW and TSW.

Methodology: A duplex real-time RT-PCR assay for the detection of the HCV-RNA and an internal control in DBS was set up, and its analytical and clinical performance was assessed. The acceptability, feasibility, and yield of HCV testing at this centre were assessed in comparison with OraQuick HCV Rapid Antibody testover one year. Epidemiological and behavioural data related to HCV infection were collected.

Results: The molecular assay showed a lower limit of detection of 541 IU/mL, and was precise and reproducible. DBS were demonstrated to be stable at room temperature for at least 2 months. The assay was 100% sensitive and specific in comparison with our frontline viral load assay (Abbott Molecular). Acceptability of HCV testing was very high (95.4%) among the 631 individuals approached. Four MSM were excluded as they had a previous HCV diagnosis (0.64% self-reported seroprevalence), and another four were underage. Among the 594 participants, 73.6% were MSM,

10.2% MSW and 16.2% TSW. All rapid antibody tests were negative. Among the 653 DBS collected (59 individuals were tested 2–3 times), invalid results due to sample collection or nucleic acid extraction were obtained in 26 cases (3.98%). HCV-positive results were obtained in five individuals, who were asked to get a confirmatory test in venous blood; one did not answer, another was negative upon DBS retesting (did not wish to have a venipuncture) and the other three viral loads were undetectable. Thus, a 0.64% false-positivity rate was obtained, and confirmed to be due to unspecific amplification. Focusing on sexual behaviours, 31.8% reported condomless receptive anal intercourse with non-steady partners, 41.3% group sex, and 58% the use of recreational drugs before or during sex. Regarding STIs, 6% were HIV positive, gonorrhoea was especially prevalent in MSW (19.1%) and syphilis in both MSW (20.7%) and TSW (23.9%).

Conclusions: HCV testing was easily implemented and well accepted. Data suggests that regular HCV screening of HIV-negative MSM is not justified. Nevertheless, given the observed high-risk behaviours and the presence of other STIs, HCV spread among MSM, MSW and TSW should be closely monitored. The molecular assay showed a good performance in DBS, although a small number of false-positive results was obtained in line with the very low HCV prevalence observed. This assay is currently being evaluated in higher prevalence groups, such as injection drug users.

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Abstract no: 260
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Clinical evaluation of the Aptima HBV Quant assay for hepatitis B virus DNA quantification on Panther system

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Background: The Hologic Aptima HBV Quant assay (Aptima HBV) is a new real-time transcription-mediated amplification method for use in monitoring of HBV infection that runs on the fully automated Panther system in random access. Here we describe validation studies of the assay compared to the Cobas AmpliPrep/Cobas TaqMan HBV Quantitative Test, v2.0 (Roche HBV) in a clinical routine setting.

Methods: For method comparison 387 prospective (274) and retrospective (113) clinical plasma samples from HBV infected patients were tested side-by-side in Aptima HBV and Roche HBV to analyze concordance on qualitative results as well as correlation between quantitative results. Precision was demonstrated by testing plasma dilutions of the AcroMetrix HBV High Control (#960603, Thermo Fisher Scientific) in 3 different target concentrations, 500, 100, and 25 IU/ml, and tested in replicates of 21 each with the respective assays. Repeatability across different genotypes was demonstrated with the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for NAAT-Based Assays (PEI code 5086/08), at a nominal target concentration of 1000 IU/ml, in three replicates for each genotype. Linearity was assessed by preparing serial dilutions of 4 well characterized clinical samples (GT A, GT C, GT D, GT E), with six dilution levels and target concentrations at 7 log IU/ml, 6 log IU/ml, 5 log IU/ml, 4 log IU/ml, 3 log IU/ml, 2 log IU/ml. Five replicates of each dilution level were tested side-by-side in the Aptima and Roche assays over 3 days, respectively. Accuracy was tested based on a Qnostics HBV Evaluation Panel (QNCM14-038-HBV).

Results: With prospective clinical samples ($n = 274$), inter-assay agreement between Aptima HBV and Roche HBV for qualitative results was substantial (79.9%). The concordance between results for 123 prospective and 113 retrospective samples with quantitative results in both assays was very high. Aptima HBV reported slightly higher values by an average of just 0.02 and 0.03 $\log_{10}$ IU/mL, calculated according to Bland–Altman method, respectively. Linear regression gave a Pearson r concordance correlation coefficient of 0.96 ($P < 0.0001$) for prospective samples and 0.98 ($P < 0.0001$) for retrospective samples. Both assays showed excellent linearity ($r > 0.99$) across four different serial dilutions of clinical samples representing different genotypes (GT A, C, D, E), with regression lines nearly parallel to the identity lines for both assays. Precision was 0.19 log SD or lower across the dilutions levels with the Acrometrix standard and comparable to Roche. The Aptima HBV demonstrated excellent accuracy in the Qnostics HBV EQA panel and proper genotype detection based on the PEI GT panel, with Aptima result differing not greater than ±0.5 log IU/ml from nominal target values based on Abbott RealTi*m*e HBV and the comparator assay, Roche HBV, respectively.

Conclusion: The Aptima HBV demonstrated excellent precision, linearity, and accuracy in all genotypes tested. Excellent concordance was observed between Aptima HBV and Roche HBV when testing clinical samples from patients on therapy. This study has demonstrated that Aptima HBV meets the necessary requirements for HBV viral load monitoring in a clinical routine setting.

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Abstract no: 261
Presentation at ESCV 2016: Poster 108

Evaluation of a novel assay for hepatitis C virus genotype 1 subtyping

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Background: Due to its high genomic variability, accurate HCV genotyping and genotype 1 subtyping remains challenging as studies have reported limitations for sequencing, line probe and PCR commercial assays. In a few cases, the RealTi*m*e HCV Genotype II assay (GT II, Abbott Molecular) does not assign the genotype 1 subtypes 1a or 1b (based on the NS5B region). Use of a second assay can further characterize those ambiguous results in order to provide clinicians with the required information for making treatment decisions.

Aim: The performance of the new HCV Genotype *Plus* RUO assay (GT Plus, Abbott Molecular) was evaluated in comparison with a reference method (sequencing and phylogenetic analysis of the NS5B and core regions) in a set of specimens previously tested by the GT II assay.

Methodology: The following plasma or extracted RNA specimens previously tested by the GT II assay were studied ($n = 66$): (i) 45 genotype 1 specimens with no subtype assigned; (ii) 10 spec-

imens identified as subtype 1a; (iii) 11 specimens identified as subtype 1b. All specimens were tested with the GT *Plus* assay on the *m*2000 RealTi*m*e System. This assay targets the core region to specifically identify subtypes 1a and 1b as well as genotype 6 and is designed to be used as a reflex test if the GT II assay fails to subtype genotype 1 (based on the 5′UTR and NS5B regions). The reference method (RT-PCR and sequencing of the core and NS5B regions followed by phylogenetic analysis) was performed in 49 specimens either without assigned subtype by the GT II assay, with discrepant subtype assignment between the two Abbott assays or with a "not detected" result by the GT *Plus* assay.

Results: Among those specimens without assigned subtype by the GT II assay, the GT *Plus* assay was able to subtype 38 cases (84.4%), while the other 7 cases (15.6%) were not detected. Among those specimens identified as 1a by the GT II assay ($n = 10$), 9 (90%) were concordantly identified by the GT *Plus* assay, while one specimen was identified as having a mixed infection (1a + 1b). Among those specimens identified as 1b by the GT II assay, 8 (72.7%) were concordantly identified by the GT *Plus* assay, while three (27.3%) were not detected. All viral loads were above the lower limit of detection of the assay (range, 2.96–7.84 log(IU/mL)). Two specimens corresponding to subtypes 1e ($n = 1$), and 1 g ($n = 1$) showed "not detected" results in concordance with the GT *Plus* assay design. Another 8 "not detected" specimens (8/66, 12.1%) were classified as 1b. Among the rest of specimens, all subtype assignments by the GT *Plus* assay were in agreement with HCV classification by the reference method (NS5B and core regions, confirming the absence of recombination events) except for one specimen (97.4%) where a mixed subtype infection was detected by the former assay (1a + 1b) that could not be evidenced by Sanger sequencing.

Conclusions: The GT *Plus* assay was able to subtype 84.4% of the specimens not subtyped by the GT II assay and showed a good agreement (97.4%) with the reference method for the identification of 1a and 1b subtypes. Sequences generated in this study may help to understand the reasons for not detected results among certain 1b isolates.

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Abstract no: 265
Presentation at ESCV 2016: Poster 109

Prevalence of hepatitis delta virus infection among hepatitis b virus surface antigen positive patients diagnosed in a Central Hospital in Portugal, a 5 years retrospective study

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Background and aims: Hepatitis delta virus (HDV) is a single-stranded, circular RNA virus that requires the hepatitis B surface antigen (HBsAg) to infect. A dual HBV-HDV infection progresses rapidly, and is associated with more complications: cirrhosis, end-stage liver disease, and hepatocellular carcinoma when compared with HBV monoinfection. HDV is transmitted by parenteral exposure and the basis of the diagnosis is the presence of antibodies against HDV (anti-HDV) and hepatitis B surface antigen (HBsAg) in the serum of a patient with chronic liver disease. It is confirmed by the presence of the HDV antigen.

The hepatitis D prevalence vary between geographic regions, but it remains endemic in many regions of the globe. The Health Care effective measures to control HBV infection have consistently diminished the circulation of HDV in countries where they were applied (vaccination + prevention). However, recent studies report a rising prevalence of HDV infection attributed to immigration from high prevalence areas and to local groups of intravenous drugs users.

The aim of our study was to characterize hepatitis delta virus infection prevalence among hepatitis B virus surface antigen positive patients diagnosed in a Central Hospital in Portugal in the last 5 years (2011–2015).

Results: From 2011 to 2015, our Laboratory followed 1998 patients with detected HBsAg (1158 male, 840 female, mean age 44.9 [2–94 years old]). We were requested to study 2071 patients for HDV infection (1140 male, 931 female, mean age 47.8 [1 month–98 years old]).

86 patients tested positive for acute or previous HDV infection (AgHVD and/or AcHVD IgM and/or AcHVD IgG positive): 61 male, 25 female, mean age 42.4 [19–72 years old]. These patients were followed mainly in Clinical Services of Gastroenterology (45) and Infectious Diseases (28).

We found 41 patients with acute HVD infection (2 patients AgHVD positive; 1 patient AgHVD/HVD IgG positive; 1 patient AgHVD/HVD IgM/HVD IgG positive; 3 patients HVD IgM positive; 34 patients HVD IgM/IgG positive) and 45 patients with previous HVD infection (HVD IgG positive).

Conclusions: Precise rates of prevalence and risk factors must be available to help clinicians decide who to screen. Because of the more aggressive nature of HBV-HDV dual infection the diagnosis and beginning of an adequate treatment regímen should not be delayed.

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Clinical evaluation of the Veris HCV assay for hepatitis C virus RNA quantification

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Background: Accurate detection and quantification of hepatitis C virus (HCV) RNA is essential for treatment monitoring but also for viral safety of blood and organ donation. Molecular diagnostics platforms are evolving towards reduced and simplified handling procedures such as the fully automated, random access DxN VERIS MDx System (Beckman Coulter). The clinical performance of the Veris HCV assay was evaluated in comparison with the RealTime HCV assay (Abbott Molecular).

Materials and methods: The panel comprised 286 plasma specimens from 146 HCV-positive patients, including 142 serial samples from 25 patients (3–11 time-points/patient) on treatment monitoring of whom 20 achieved sustained virological response (SVR) and 5 experienced relapse. Quantitative results were compared by Bland–Altman method.

Results: Indeterminate Veris results (PCR inhibitors) were obtained in 6 cases (2.1%). In the remaining 280 samples, all 54 Abbott undetectable results returned undetectable; from 46 samples with detectable HCV RNA below 12 UI/ml, 42 (91.3%) returned not detected and 4 returned viral loads ranging from 1.32 to 1.73 log UI/ml; among 180 samples with Abbott results within the

1.08–8 log UI/ml range, 10 (5.5%) with viral loads ranging from 1.18 to 1.87 log UI/ml were missed by the Veris assay and 1 with a 7.97 log UI/ml returned >8 log UI/ml. Quantification bias was calculated for the remaining 169 samples (67 gt1, 17 gt2, 33 gt3, 46 gt4, 2 gt5, and 4 gt6), the mean bias (Abbott–Veris) was 0.087 log UI/ml (CI −0.894 to 1.068), with <0.5 log = "0" = "5-1" and =>1 log UI/ml difference in 69.9%, 26.6% and 3.5% of cases, respectively. The maximal bias was observed for genotype 4 samples (0.572; CI −0.287 to 1.430), where an underestimation of viral load of 0.5–1.5 log UI/ml was observed in 56.5% of cases. Regarding treatment monitoring, HCV RNA remained detectable at low levels by the Abbott assay 1 to 3 time-points after Veris negativation in 17/20 patients who achieved SVR; relapse was detected earlier by the Abbott assay in 2/5 cases.

Conclusions: Discordances in HCV RNA detection between the Veris assay and the Abbott assay were observed for low viral loads, with little or no impact on patient monitoring in the present study. However, significant underestimation of genotype 4 samples raises concerns and requires optimisation of assay design for this highly variable genotype.

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Abstract no: 271
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Reconstruction of the regional transmission of HCV in Southern Sweden by phylodynamics

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Background: The molecular epidemiology studies of HCV are useful to gain in-depth knowledge of timing and patterns of viral spread. Analysis of laboratory testing sequence data can give insights for prevention of virus spread. The objectives of the current project were to characterize the genetic diversity and transmission dynamics of HCV in Southern Sweden, as well as identifying socio-demographic variables that are associated with onward transmissions.

Methods: Partial HCV *NS5B* gene sequences (339 bp) were available from routine clinical testing for genotyping of clinical isolates. The sequences were collected between 2004 and 2015. Sub-genotyping was based on similarity search using Los Alamos HCV sequence database BLAST tool. Phylogenetic analysis using maximum likelihood method (ML) was done based on Swedish and reference sequences retrieved from GenBank using BLAST tool. ML trees were reconstructed for determination of transmission clusters representing domestic spread of HCV in Sweden. Analysis of the transmission clusters was done using Cluster Picker software with monophyletic Swedish clades defined as those having approximate Likelihood Ratio Test SH like (aLRT-SH) support value of 0.90 and containing more than 70% Swedish sequences. Transmission clusters with two sequences were defined as dyads, those containing between three and 14 sequences were defined as networks and those having more than 14 sequences were defined as large clusters. Estimation of the evolutionary rate and time to most recent common ancestors (tMRCAs) of the large Swedish clusters were determined using Bayesian approach in BEAST.

Results: A total of 3912 sequences with Open Reading Frames (ORFs) were available for analysis. Genotyping revealed the following distribution: 1a (39%, $n = 1513$), 3a (38%, $n = 1481$), 2b (10%, $n = 390$) and 1b (10%, $n = 374$). For sub-genotype 1a, 550 sequences were part of 138 transmission clusters (36%). Those clusters were classified as 83 dyads, 50 networks and five large clusters. Possible geographic origins of some clades that resulted in domestic spread of sub-genotype 1a included: Cyprus, Greece, Ireland, Iran, Netherlands and USA. For sub-genotype 3a, 698 sequences were part of 142 transmission clusters (47%). Those clusters were classified as 73 dyads, 60 networks and nine large clusters. Possible geographic origins of some clades that resulted in domestic spread of sub-genotype 3a included: Canada, Spain, Brazil, Malaysia, Switzerland, China, Uzbekistan, Iran and Netherlands. For sub-genotype 2b, 178 sequences were part of transmission clusters (46%). Those clusters were divided into 36 dyads, 21 networks and one large cluster. For sub-genotype 1b, 43 sequences were part of transmission clusters (11%) and were classified into 17 dyads and two networks. The time to Most Recent Common Ancestor (tMRCA) of the oldest sub-genotype 1a cluster dated back to 1967 (95% HPD: 1959–1975). The tMRCA of the oldest sub-genotype 3a cluster dated back to 1969 (95% HPD: 1962–1976).

Conclusions: Four HCV sub-genotypes were prevalent in Southern Sweden between 2004 and 2015, with sub-genotypes 1a and 3a dominating the infections in terms of prevalence. The phylodynamic approach unravelled patterns of viral spread and possible geographic origins of HCV in Southern Sweden. Further studies including association of socio-demographic variables to members of clusters are underway and might be helpful in implementing strategies for infection control.

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Occupational exposure to hepatitis E virus (HEV) in Portuguese swine workers

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Introduction: The concept of zoonotic hepatitis E has emerged with the discovery of animal strains of hepatitis E virus (HEV) closely related to human HEV. Today, pigs are recognized as major reservoirs and sources of HEV infection in humans. Different routes of zoonotic HEV transmission have been recognized such as the consumption of undercooked or raw meat from infected swine as well as the contact with infected pigs. Many studies have reported a high seroprevalence of anti-HEV in swine slaughterhouses workers when compared with the general population, suggesting that this professional group may be at higher risk for HEV infection.

Aims: The aim of this study was to investigate if there was also an occupational risk of zoonotic HEV infection in Portuguese swine slaughterhouse workers based on the differences of anti-HEV seroprevalence rates between these professionals and the general

Portuguese population. We also aimed to search for recent HEV infection in slaughterhouse workers. This study was funded by EEA grants and is part of HEPeCONTROL project (60DT2).

Materials and methods: A case–control study was conducted among swine slaughterhouse workers in Portugal. Serum samples ($n = 114$) were collected from swine workers of slaughterhouses of the North and Centre of Portugal between September and November 2015. Sera ($n = 804$) from the general population matched by age, gender and region, obtained from anonymous volunteers were used as controls. All samples were tested for anti-HEV IgG and IgM antibodies by an enzyme immunoassay (recomWell HEV IgG/IgM version 2015, Mikrogen, Germany) and retested by immunodot assay (recomLine HEV IgG/IgM version 2015, Mikrogen, Germany) if positive for anti-HEV IgM. A chi-square test for homogeneity of proportions was used to determine significant differences in anti-HEV prevalence between study groups.

Results: Anti-HEV IgG was found in 30.7% (35/114) of workers and in 19.9% (160/804) of general population. Differences between these two groups showed to be statistically significant ($p < 0.05$). Two workers presented only anti-HEV IgM without anti-HEV IgG suggesting recent HEV infection.

Discussion: The results obtained in this study showed that Portuguese swine slaughterhouses workers have higher HEV seroprevalence than the general population. Therefore, these professionals should be considered a specific risk group for HEV infection and hygiene measures are highly recommended to reduce their exposition to HEV. This study is the first in Portugal focusing on the occupational risk of swine workers of slaughterhouses.

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Clinical and analytical performance characteristics of the VERSANT HCV RNA 2.0 (kPCR) assay

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Hepatitis C viral (HCV) RNA detection and quantitation are of central importance for confirmatory diagnosis and monitoring of chronically-infected HCV patients after infection and while undergoing treatment. The new VERSANT® HCV RNA 2.0 (kPCR) Assay* has been designed to support both of these applications simultaneously on a single assay platform. Utilizing a dual target/dual amplicon assay design, the assay is intended to achieve good sensitivity, specificity and standardization across diverse HCV genotypes. The objective of this study is to assess the analytical performance of the new VERSANT HCV RNA 2.0 (kPCR) Assay, and to compare its quantitative performance to current CE-IVD HCV viral load assays with clinical samples across all six major HCV genotypes.

Analytical performance testing of the VERSANT HCV RNA 2.0 (kPCR) assay evaluated sensitivity, specificity, linearity, precision, and accuracy. Linearity was also evaluated across each HCV genotype (1a, 1b, 2a, 2b, 3, 4, 5, 6). The estimated limit of detection (PROBIT) was 7.44 IU/mL with a 95% CI of 5.4–10.23 IU/mL in plasma and 8.84 IU/mL with 95% CI of 6.6–11.74 IU/mL in serum. Linearity was evaluated across the test range (22–1,100,000 IU/mL) in both serum and plasma. The log difference between the mean quantitated value and the linearized quantitated value is within 0.12 log. The calculated between lot %CV was less than 30 for both matrices across the linear range. Likewise, the observed accuracy was within 0.2 log of the target concentration. The VERSANT HCV RNA 2.0 (kPCR) assay exhibited a specificity of 100% in both matrices. Linearity was evaluated over a range of 10–1,000,000 IU/mL for all major HCV genotypes. The log difference between the mean quantitated value and the linearized quantitated value over this range is within ±0.05 log.

A total of 236 HCV-positive samples across genotypes 1–6 were tested for viral load determination with the VERSANT HCV RNA 2.0 (kPCR) Assay and two widely-used comparator assays. Samples were evenly distributed across the common HCV genotypes/subtypes (1a: 16; 1b: 14; 2: 9; 2a-c: 11; 2b: 25; 3: 48; 4: 33; 5: 47; 6: 33). Results from the VERSANT HCV RNA 2.0 (kPCR) Assay were compared using statistical methods with matched sample test results from the Abbott Real-Time HCV Assay and the Roche CAP/CTM 2.0 Assay. Overall, the average log difference was 0.18 log when comparing the VERSANT HCV RNA 2.0 (kPCR) Assay and Abbott Real-Time HCV Assay, and −0.24 log when comparing the VERSANT HCV RNA 2.0 (kPCR) Assay and Roche CAP/CTM 2.0 Assay. When evaluated on a per genotype basis, the new VERSANT HCV RNA 2.0 (kPCR) Assay testing results consistently laid between the comparator methods, demonstrating how the Siemens assay is well standardized with resulting quantitation between the relative quantitation extremes of the Roche CAP/CTM 2.0 Assay and the Abbott Real-Time HCV Assay.

Overall these performance characteristics suggest the new VERSANT HCV RNA 2.0 (kPCR) Assay as a favorable alternative to current HCV viral load assays.

*VERSANT HCV RNA 2.0 (kPCR) Assay under feasibility evaluation. Not for sale and its future availability cannot be guaranteed.

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Presentation at ESCV 2016: Poster 114

Hepatitis A and E among asymptomatic pregnant women and acute hepatitis patients from South Tunisia

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Background: Hepatitis A and E viruses (HAV and HEV) are both non enveloped viruses responsible for enterically transmitted hepatitis. Tunisia is reported to be a country of intermediate endemicity for HAV (≥50% have immunity by age 15) [1] and of low seroprevalence for HEV (<10%) [2]. The aim of this study was to assess hepatitis A and E markers among asymptomatic pregnant women and patients with acute hepatitis in rural areas of South Tunisia.

Methods: Sera from 216 asymptomatic pregnant women undergoing routine gynecological screening and from 92 patients with acute hepatitis were collected between October 2014 and November 2015 from the hospitals in Gabes, Medenine and Tatouine town in South Tunisia. Total and IgM anti-HAV immunoglobulins and anti-HEV IgG and IgM were investigated. HAV IgM positive samples were subjected to in house RT-PCR targeting 500 nt of the VP1/2A region and sequenced. Anti-HAV IgG

avidity index was determined for HAV RNA negative samples. HEV IgM positive samples and all samples from acute hepatitis patients were assessed for HEV RNA by using a quantitative real-time RT-PCR assay.

Results: Among asymptomatic pregnant women (mean age 32 ± 8), HAV seroprevalence was 98.61%, none presented anti-HAV IgM and HEV seroprevalence was 5.09% and three (1.38%) presented weakly reactive anti-HEV IgM without detectable HEV RNA. Among acute hepatitis patients (mean age 18.5 ± 14), HEV seroprevalence was 20.65%, none presented anti-HEV IgM. HAV seroprevalence reached 95% by the age of 5 and HAV IgM was detected in 23 patients (25%) aged 11.7 ± 26.9. HAV RNA was negative high anti-HAV avidity index demonstrating past infection in 3/22 available samples. Detectable HAV RNA confirmed HAV infection in 19/22 available samples. Confirmed HAV cases represented 52%, 10%, and 7% of acute hepatitis in Gabes, Medenine and Tatouine, respectively. Phylogenetic analysis identified genotype IA strains with a 1st cluster responsible for 10/13 cases in Gabes, a 2nd responsible for 5/5 cases in Medenine, the remaining cases being associated with unique strains.

Conclusion: The present study confirmed low HEV endemicity and evidenced a high level of HAV circulation in South Tunisia probably associated with inadequate wastewater treatment.

Reference

[1] Jacobsen, et al., Hepatitis A virus seroprevalence by age and world region, 1990 and 2005, Vaccine 28 (41) (2010) 6653–6657.
[2] Kim, et al., A systematic review of the epidemiology of hepatitis E virus in Africa, BMC Infect. Dis. 14 (2014) 308.

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Abstract no: 297
Presentation at ESCV 2016: Poster 115

Detection and genetic characterization of imported hepatitis E virus genotype 1 of probable Indian origin, Portugal, 2016

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Introduction: Until recently hepatitis E virus (HEV) infection was thought to occur exclusively in developing countries but it is now known that locally acquired HEV is common in industrialized regions. Unlike genotypes 3 and 4 that circulate in industrialized countries, genotypes 1 and 2 of developing countries produce more severe disease and occur in the epidemic form with mortality rates ranging from 0.2% to 4.0%. These HEV genotypes are considered a serious public health problem and are known to cause an estimated 20.1 million infections with 3.4 million acute cases annually worldwide with an estimated 70,000 deaths. In the present work we report the first case of HEV genotype 1 in Portugal associated to a severe acute hepatitis in a patient that had returned from India.

Epidemiological and laboratory investigations: On January 2016, a 31-year-old Indian man was admitted to the Emergency Unit of Centro Hospitalar de Lisboa Central, Lisboa, Portugal, with clinical signs compatible with acute hepatitis. Upon admission he reported to have resided in the North of India until coming to Portugal 6 weeks earlier. Blood tests showed elevation of hepatic enzymes supporting the presumptive diagnosis of an acute viral hepatitis. Serum was tested for the hepatitis virus panel and was negative for all viruses, however HEV markers were not searched for. Instead, RT-qPCR detection for HEV RNA in sera using generic primers/probe targeting the open reading frame (ORF) 2 region [1] was performed confirming the presence of HEV RNA. HEV sequence was characterized using a nested broad-spectrum reverse transcription PCR with amplification within the ORF 1 [2] followed by phylogenetic analysis.

Results: Genetic characterization indicated that the virus isolated from this patient belonged to genotype 1, clustering with HEV genotype 1 sequences from India and Nepal retrieved in 2013 and 2014. In particular, sequence identity matching with the isolated virus showed that it shared the highest nucleotide identity (96.0%) with sequences isolated in 2013 and 2014 in Jabalpur District, India, and from an HEV outbreak in Nepal, 2014.

Discussion: In this case report we describe a patient presenting an acute hepatitis E caused by HEV genotype 1 most likely acquired during his stay in India. Our findings demonstrate the need to implement and improve strategic HEV surveillance in countries with substantial migration flows.

Reference

[1] K.J. Rolfe, M.D. Curran, N. Mangrolia, W. Gelson, G.J. Alexander, M. L'estrange, R. Vivek, R. Tedder, S. Ijaz, First case of genotype 4 human hepatitis E virus infection acquired in India, J. Clin. Virol. 48 (1) (2010) 58–61.
[2] R. Johne, A. Plenge-Bönig, M. Hess, R.G. Ulrich, J. Reetz, A. Schielke, Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR, J. Gen. Virol. 91 (Pt 3) (2010) 750–758.

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Abstract no: 300
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Preliminary study of the prevalence of hepatitis E virus infection in liver transplant recipients in Portugal

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Background: *Hepatitis E virus* (HEV) is classified within the genus *Hepevirus* of the *Hepeviridae* family. HEV has caused a significant number of acute hepatitis outbreaks in developing countries which have been associated with contaminated water transmission. In contrast, sporadic autochthonous infections in developed countries have been mainly associated with zoonotic strains with

likely major reservoirs in domestic pigs, wild boars and deer. HEV infections have been associated with self-limiting hepatitis. However, an increasing number of chronic infections have been described in immunosuppressed individuals, such as organ transplant recipients, in whom HEV infection can cause progressive liver fibrosis, cirrhosis and subsequent liver failure, which is particularly relevant for liver transplant recipients. The aim of this study was to assess the prevalence of HEV infection in liver transplant recipients in a Portuguese reference center for liver transplantation.

Methods: A total of 23 individuals (children and adults) transplanted between 2003 and 2015 was assessed for evidence of HEV infection by testing post-transplant plasma samples for HEV RNA. Additionally, previous and subsequent plasma samples were also tested in all individuals with evidence of HEV infection to evaluate the possibility of chronic infection (defined by persisting HEV RNA in plasma for 6 months or more). Epidemiologic and clinical data at the time of sample collection were recorded. Viral RNA was extracted from plasma samples using the QIAamp Viral RNA Mini Kit (Qiagen) and amplified by real time RT-PCR using an assay targeting the ORF2/3 overlapping region (Ceeram).

Results: Median age at the time of liver transplant was 28 years old and median time since transplantation to sample collection was 69 months. Median value of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) at the time of sample collection was 54 U/L and 53 U/L, respectively. Overall, 8.7% ($n = 2$) of the individuals had evidence of post-transplant HEV infection. Of these, one individual had detectable HEV RNA in plasma, between months 79 and 80 post-transplant, and median value of ALT and AST at the time of positive samples was 198 U/L and 99 U/L, respectively. This individual underwent antiviral therapy with ribavirin and successfully cleared the virus. The other individual had detectable HEV RNA in plasma 113 months after transplant, which persisted for 13 months, and therefore, was considered as chronically infected. Median value of ALT and AST at the time of positive samples was 68 U/L and 60 U/L, respectively. A reduction in immunosuppression (tacrolimus) was attempted to clear the virus, however, sustained viral clearance was only achieved after antiviral therapy with ribavirin.

Conclusion: Although the overall prevalence of HEV infection was relatively low (8.7%), the results of this study demonstrate that liver transplant recipients in Portugal have a risk for HEV infection. Supporting this evidence is the recently described high prevalence of HEV in domestic pigs and wild boars in Portugal, which have been described as the most likely sources of infection in developed countries. Moreover, to our knowledge, this study describes the first case of chronic HEV infection in liver transplant recipients in Portugal. In conclusion, HEV screening should be considered in the liver transplant setting, particularly in the differential diagnosis of graft hepatitis of an unclear etiology.

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Abstract no: 301
Presentation at ESCV 2016: Poster 117

The frequency of occult HBV infection in Eskisehir region of Turkey between 2001 and 2015

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Occult HBV infection (OBI) is characterized by the detection of HBV DNA in low levels in serum and peripheral blood mononuclear cells and/or in the liver, in the absence of detectable hepatitis B surface antigen (HBsAg). The prevalence of occult HBV infections varies among patient populations tested. It depends on the prevalence of HBV infection in populations, sensitivity of the assay employed in routine serological or nucleic acid test screening and also the nature of biological material tested.

In this study, we searched the presence of occult HBV infection in patients diagnosed as viral hepatitis B infection. A total of 16853 serum samples were tested for HBV DNA by Real-time PCR technique and for serological viral markers (HBV, HCV and HDV) by ELISA assay (*AxSYM* and Architect İ2000SR (Abbott) between 2001 and 2015. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were investigated in the sera of HBV DNA positive and HBsAg negative patients. We detected HBsAg negativity in 105 (2.6%) of 4036 patients, of whom HBV DNA was positive. The minimum and maximum DNA levels were as 1×10^1–1.7×10^8 copies/ml. Anti-HBc IgM was negative in all of these 105 patients. Among 105 patients, 31 (29.5%) were positive for only anti-HBc, 3 (2.8%) were positive for anti-HBs, 16 (15.2%) were positive for both anti-HBs and anti-HBc in their sera. Thirteen (12.3%) of all patients were negative for serological markers of HBV infection. Among 105 patients, five patients were anti-HCV positive. All of the patients were negative for anti-HDV. Forty (38%) and thirty-eight (36.1%) patients had abnormal ALT and AST levels; respectively. Nineteen (18%) patients were immunocompromised individuals.

Detection of HBV DNA with highly sensitive and specific PCR techniques is important because OBI is usually associated with low levels of HBV DNA. OBI should be carefully assessed in certain clinical statements: HBV infection transmission (via blood transfusion or solid organ transplantation), liver disease progression, hepatocellular carcinoma onset, and HBV reactivation.

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Abstract no: 317
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Anti-HAV IgG seroprevalence in Lisbon region residents: Preliminary results from the National Serological Survey 2015–2016

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Background: Data from developed countries have shown a decrease in hepatitis A virus (HAV) incidence over time as a consequence of economic and sanitation levels improvement. WHO classifies Western European region, where Portugal is included, as a low endemicity area for hepatitis A. The presence of immunoglobulin G (IgG) antibodies to HAV in serum is a marker of past infection or vaccine immunization and is used to assess seroprevalence.

The 3rd National Serological Survey 2015–2016 (NSS), funded by Iceland, Liechtenstein and Norway through the EEA Grants, was designed to study the immunity of Portugal's residing population for vaccine-preventable diseases. Although it has never been included in the National Vaccination Programme, hepatitis A vaccine is available in Portugal since 1998.

Aim: To present, as preliminary results of the NSS, the seroprevalence of IgG antibodies to hepatitis A virus in a population-based cross-sectional study of Lisbon region residents.

Materials and methods: Sample size was estimated based on seroprevalence data from the previous NSS, sample has been equally distributed by gender and assuming at least a precision of 5% and a design effect of 1.5. Serum samples from 304 participants, 153 of them females (50.3%), aged 15 years or more, resident in NUTII area geographically corresponding to Lisbon region, were tested for IgG antibodies to HAV (anti-HAV IgG), using ARCHITECT HAVAb-IgG™ (Abbott), a chemiluminescent microparticle immunoassay (CMIA). Study participant's demographic data were collect using a questionnaire and were registered in a database. Statistical analysis was performed using the Chi-square test with a significance level of 5%.

Results: Anti-HAV IgG antibodies were detected in 149 samples representing an overall seroprevalence of 49.0% (CI 95%: 43.3–54.8). Stratification by gender, age group and country of birth showed no significant difference in seroprevalence distribution by sex (male = 53.6% *vs* female = 44.4%; $p = 0.136$). By age group the seroprevalence ranged between 30.1% for 20–29 y/o age group to 87.5% for ≥55 y/o age group ($p < 0.001$); a higher seroprevalence was observed in individuals born outside WHO European region ($n = 15$; 12/15 born in African countries) – 80.0% *vs* 46.6% ($p = 0.024$).

Discussion and conclusion: Our results are in accordance with previous NSS findings and other published studies from same geographical region. A high seroprevalence of anti-HAV IgG in older individuals was expected and is explained by a more extended period of virus exposure, including infancy and youth lived in a period of higher hepatitis A incidence. Although tests to detect anti-HVA IgG do not differentiate between post infection and vaccine induced antibodies, vaccination rate is not expected to be high in adults. Information concerning the low immunity rates at younger ages and consequent susceptibility to HAV infection is of particular relevance in times were travelling to highly endemic areas is more frequent. In most of African countries HAV incidence is known to be high which explains the findings for Africa born participants. Gender similarity for HAV antibodies prevalence has been consistently described showing a similar transmission pattern.

The NSS 2015–2016 is still ongoing and samples from further regions and age groups are being collected and studied. At the end this study will enable to establish Portugal's resident population immunity/susceptibility profile for HAV.

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Abstract no: 331
Presentation at ESCV 2016: Poster 119

Single-step hepatitis C testing: Simplifying the clinical pathway from primary care to specialist services

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Background: Direct acting antivirals have revolutionised treatment opportunities for patients with hepatitis C virus (HCV) infection. Accessing treatment requires timely diagnosis but prevalent laboratory testing algorithms in the United Kingdom require two consecutive blood draws (1) serum for anti-HCV antibodies (2) whole blood for HCV RNA. This 2-step process may increase attrition rates in the clinical pathway for HCV-infected patients. At the same time, recent advances in laboratory methods now enable RNA extraction directly from serum making single-step testing on one blood draw possible.

Objective: To compare 2-step versus single-step HCV testing in terms of completion of testing algorithm and referral rates to specialist services.

Design: Uncontrolled before and after study of patients tested for HCV infection in primary care (December 2013 to April 2016). From 1 March 2015 samples with a first detection of anti-HCV antibody were simultaneously tested for HCV RNA.

Results: The prevalence of anti-HCV antibody positivity was similar throughout the study period (3.1% (141/4525) during 2-step testing and 2.8% (115/4151) during single-step testing). Completion of HCV testing for antibody positive samples with RNA confirmation was 70% with 2-step testing while only a single specimen was inhibitory in single-step testing. The overall proportion of patients with detectable HCV RNA was 53% (112/213) of whom 13% (15/112) were previously known diagnoses. For those with a first diagnosis of active HCV infection, although there was no statistical difference in referral rates to specialist services (88% (44/50) in single-step testing versus 92% (43/47) in 2-step testing, $P = 0.55$) there was a trend towards shorter time from first blood draw to specialist assessment with single-step testing (median time 80 days (interquartile range, IQR 63–116) versus 140 days (IQR 56–272), $P = 0.06$). In addition there were fewer unnecessary referrals to specialist services for patients with no evidence of active infection although this difference was not statistically significant (6% (3/53) in single step testing versus 16% (7/43) in 2-step testing, $P = 0.09$).

Conclusions: Referral rates for specialist assessment and treatment are high once diagnosis of active HCV infection is made. However, 30% of HCV antibody-positive patients have unknown infection status. This attrition rate from missed diagnoses is eliminated by single-step testing. Based on this quality improvement, avoidance of repeat blood draws for patients and cost-savings from unnecessary specialist assessment single-step HCV testing should be standard of care in the HCV clinical pathway.

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Presentation at ESCV 2016: Poster 120

HEPATITIS E – Reaction or unspecific reaction?

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Background: Hepatitis E (HEV) is not endemic in Denmark, yet a substantial proportion of HEV patients have no travel history. Most Danish HEV cases are serologically diagnosed. Especially among elderly patients with competing diseases, the clinical interpretation can be difficult if a simultaneous fecal sample is PCR negative. Reactivation among transplantation patients is well recognized while possible recurrence among immunocompetent patients is still debated [1,2].

Question: We want to identify if patients presently investigated for HEV have encountered HEV earlier in life, and whether the current reaction can be due to a reactivation of the virus? To answer the question concerning reactivation, we will test a number of sam-

ples previously taken from patients presently under investigation for HEV antibodies.

Materials and methods: Some patients have blood samples taken earlier in life for other routine diagnostics than HEV, and therefore it is possible to compare the former and the present sample, for determination of the HEV status of the patient, and to conclude if it is a new infection or a reactivation.

We are planning to test approximately 20–30 patients all having a present HEV sample and a sample taken earlier in life for other reasons than HEV. All samples will be tested in three different commercial HEV elisa assays, one from Wantai, one from Mikrogen and one from DSI/Abia, and the results will be compared.

Clinical information is available for some patients and we will try to retrieve it for the rest of the patients.

Conclusion: The project is ongoing, but the final data will be ready for the conference.

Reference

[1] P. Grewal, S. Kamili, D. Motamed, Chronic hepatitis E in an immunocompetent patient: a case report, Hepatology 59 (1) (2014) 347–348.
[2] N. Kamar, J. Izopet, Does chronic hepatitis E virus infection exist in immunocompetent patients? Hepatology (2013).

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Abstract no: 342
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Hepatitis B surface antigen expression: A pilot study comparing wild type and surface antigen mutant viruses

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Introduction: The immunogenic region of the hepatitis B surface antigen (HBsAg), the 'a' determinant, is formed from a sequence of amino acids, which through disulphide bonding form a three dimensional structure. Mutations in this coding region can lead to amino acid substitutions resulting in a conformational change in the protein, which may render it unrecognisable to immunoglobulins.

By creating a construct containing the PreS1/PreS2/S region of the hepatitis B virus (HBV) genome coding for the three envelope proteins the effects of mutations in the S gene on the expression of HBsAg from transfected cells can be investigated [1].

Materials and methods: Five HBV samples were investigated: a wild type, two G145R HBsAg mutants, and two with T118K, P120S and M133T, and M133T, P135L, G145R and V168A surface mutations respectively. Nested PCR was used to obtain a 0.7x HBV genome (0.7mer) construct containing the PreS1/PreS2/S coding region.

The TOPO-TA cloning kit (ThermoFisher) was used to clone the constructs prior to transferring them into a mammalian expression vector.

Chemical transfection of HepG2 cells was performed with the FuGENE® HD transfection system (Promega®) using both 0.5 μm and 2.0 μm of DNA of the recombinant expression vector. Cells were harvested 72 h post-transfection. Supernatant fluid and cell pellets were collected. Cells grown on coverslips were fixed and labelled for microscopy.

Enzyme-linked immunosorbent assay (ELISA) for HBsAg (Murex) was used to detect HBsAg in cell pellets and supernatant fluid. Coverslips were labelled with antibodies to HBsAg, golgi and nuclear proteins for confocal microscopy.

Preliminary results: Using more DNA in the transfection resulted in higher readouts from the ELISA.

For the 0.5 μm DNA transfections, two of the mutant viruses demonstrated HBsAg secretion deficiency compared with wild type virus. This occurred in one of the G145R HBV mutants and the construct with T118K, P120S and M133T surface mutations, both of which had higher HBsAg levels in the cell pellet than the supernatant fluid. The two other viruses appeared to behave like wild type virus. For the 2.0 μm DNA transfections, however, these differences in secretion were not reflected, with samples demonstrating higher amounts of HBsAg in the cell pellet than the supernatant fluid.

Immunofluorescence showed variation in surface antigen labelling within the HepG2 cells. The cells transfected with one of the G145R mutant viruses demonstrated greater labelling within cells than those transfected with wild type and the other mutant viruses.

Conclusions and further work: The preliminary results of this pilot work indicate the presence of phenotypic differences between hepatitis B viruses with surface gene mutations. These mutations appear to have varying effects on the secretion of HBsAg from transfected cells. However, it is difficult to characterise these differences because of the contradictory results when higher amounts of DNA are used for transfection. The reasons behind the discrepancy are not clear and further work to look at the reproducibility of this phenomenon is needed.

Further phenotyping work using a Luminex® bead based assay looking at variations in specific epitopes of the HBsAg will be undertaken.

Reference

[1] T. Garcia, et al., Drastic reduction in the production of subviral particles does not impair hepatitis B virus virion secretion, J. Virol. (2009).

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Abstract no: 35
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Comparison of immunoassays from three chemiluminescent automated systems for the detection of hepatitis B virus serological markers

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Background: HBV infection is a serious global health problem. More than 350 million people suffering from chronic infection what results in 500,000 to 1.2 million deaths per year [1]. In Denmark the disease is relatively rare – the prevalence in the adult population estimated per 31st December 2007 was 0.24% [2]. Serological markers of hepatitis B virus (HBV) are used for laboratory diagnosis and monitoring of HBV infection or immune status. Hepatitis B surface antigen (HBsAg) is the hallmark of HBV infection and is the first serological marker detectable in serum, while antibodies to HBsAg (anti-HBs) can be formed following a hepatitis B infection or after hepatitis B vaccination. Hepatitis B core antibodies (IgM and IgG) are used to follow the progression of the infection from the acute stage to recovery. Anti-HBc antibodies are sometimes present after the disappearance of the HBsAg and before appearance of anti-HBs. In these situations these antibodies serve as the primary marker for infection. The hepatitis B e antigen is the marker of viral replication, and anti-HBe is a marker of immune response to HBeAg,

and is usually associated with virus inactivation [3]. Thought hepatitis B virus infection stages are serologically clearly defined the interpretation of results is not always easy in practice. E.g. anti-HBc indicates usually exposure to HBV, but found in daily practice profile such as: 'anti-HBc only' may mean false positive result. It is very important to give clinicians as accurate results as possible, so they can assess diseases stage and apply appropriate treatment. Aim of this study was to compare the results obtained with ADVIA Centaur XP (Siemens Healthcare Diagnostics, USA) chemiluminescence immunoassays, currently used to determine hepatitis B serological markers in our lab, with the results obtained with two other automated platforms: LIAISON® XL (DiaSorin, Saluggia, Italy) and Elecsys Cobas (Roche Diagnostics, Switzerland).

Materials and methods: Serum samples (patient samples and quality specimen – UK, NEQAS) have been tasted for the qualitative or quantitative detection of 6 hepatitis B virus serological markers with assays from three chemiluminescent automated systems mentioned above.

Results: The correlation between the tests performed on LIAISON® XL and ADVIA Centaur XP (patient samples) was: 100% for HBsAg, 96.7% for anti-HBs, 100% for anti-HBcIgM, 98.4% for anti-HBc-t, 100% for HBeAg and 95.3% for anti-HBe. The correlation between the tests performed on Elecsys and ADVIA Centaur XP (patient samples) was: 98.2% for HBsAg, 95.8% for anti-HBs, 97.7% for anti-HBcIgM, 94% for anti-HBc, 99% for HBeAg and 96.6% for anti-HBe. Weak positive 'anti-HBc only' results by the ADVIA Centaur anti-HBc assay were negative by both LIASON XL and Elecsys Anti-HBc assay. Results for all quality specimen were the same by all platforms.

Conclusion: All three systems demonstrated assays suitable for routine use.

Reference

[1] D. Lavanchy, Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures, J. Viral Hepat. 11 (2) (2004) 97–107.
[2] N. Hansen, G. Hay, S. Cowan, P. Jepsen, H. Bygum Krarup, N. Obel, et al., Hepatitis B prevalence in Denmark – an estimate based on nationwide registers and a national screening programme, as on 31 December 2007, Euro Surveill. Bull. Eur. Sur. Mal. Transm. Eur. Commun. Dis. Bull. 18 (47) (2013).
[3] Diagnosís of hepatitis B virus infection, 2016. www.uptodate.com.

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Comparative study of DxN VERIS molecular diagnostics system and the COBAS AmpliPrep/COBAS TaqMan platform for the determination of viral load in Hepatitis C virus infected patients

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Introduction: The ability to measure the hepatitis C virus viral load in HCV infected patients has been available for several years on molecular diagnostics platforms from a number of manufacturer's. Among these systems the COBAS AmpliPrep/COBAS TaqMan manufactured by Roche Diagnostics is widely used. The Beckman Coulter DxN VERIS system is a more recent addition to the list of platforms available for such assays. This aim of this study was to compare the performance of the DxN VERIS system with that of the Roche system for the accurate determination of HCV viral load in HCV infected patients.

Methods: A total of 71 plasma samples of patients entering our laboratory with the request for HCV viral load and HCV genotyping were collected. Samples were analyzed in parallel with the COBAS AmpliPrep/COBAS TaqMan system and the DxN VERIS system. The HCV genotype was determined using the VERSANT HCV Genotype Assay Kit (LIPA) SIEMENS.

Results: Of the71 samples tested 18 were positive and overall there was a good correlation between the two techniques ($r = 0.963$) for the parameter studied. The majority of the samples were determined to be HCV genotype 1, specifically 1a and 1b ($n = 13$), while three were un-typed. Other genotypes identified included HCV genotype 4a/4c/4d ($n = 1$) and 2a/2c ($n = 1$). Comparison of the VERIS HCV assay and Roche HCV assay results for the HCV genotype 1 sample showed that there was a good correlation between methods. This was observed for both genotype 1a or 1b. However for HCV genotype's 2a/2c and 4a/4c/4d a difference was observed between both methods (difference of −0.95 log cp/mL for 2a/2c and −1.25 log cp/mL for 4a/4c/4d).

Conclusion: The results indicate that the HCV viral load results generated on the DxN VERIS system were reproducible when compared to the reference method (COBAS). This together with the other features of the DxN VERIS system makes this device very useful in the daily routine diagnosis and monitoring of HCV viral load. The other features of the system include the ability to test samples from primary tubes and a bi-directional interface linking the DxN VERIS to the laboratory computer system (SIL). However, although it seems a coincidence, we should consider whether HCV genotypes 2 and 4 show discrepancies between the two techniques or is it a chance. A statistically significant number of samples of these genotypes should be tested to determine whether or not there is a discrepancy between the two methods. If this is confirmed then the cause should be determined.

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Abstract no: 40
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Apolipoprotein(a) inhibits hepatitis C virus entry

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In the last two decades, the development of different cell-based models has greatly contributed to improve the knowledge of HCV life cycle. However, it is still impossible to grow primary HCV isolates from each genotype in cell culture. This would open new

perspectives to investigate viral determinants responsible for the different natural course and treatment outcome of hepatitis C as well as to develop a vaccine. In this study we hypothesized that this hindrance could be due to the presence of inhibitory factors in patient serum.

Combining polyethylene glycol precipitation, iodixanol gradient and size exclusion chromatography, we obtained a purified fraction enriched in inhibitory factors from a pool of HCV seronegative serums. Mass spectrometry analysis of this fraction identified apolipoprotein(a) (apo(a)) as a potential inhibitor of the early step of HCV life cycle. Apo(a) consists of ten kringle IV-like domains (KIV), one kringle V-like domain (KV) and a protease-like domain that are homologous to plasminogen domains. Each of the ten KIV domains is present in a single copy with the exception of KIV type 2 (KIV 2), which is encoded in a variable number of tandemly repeated copies by the apo(a) gene, which gives rise to several apo(a) size isoforms in the human population. In addition, in human serum, apo(a) covalently links to the Apolipoprotein B component of a low density lipoprotein via a disulfide bridge to form a lipoprotein(a).

The inhibitory effect of apo(a) on HCV entry was confirmed using a recombinant virus derived from the JFH1 strain and supernatant of cells transfected with plasmids expressing apo(a) as well as purified recombinant isoforms of apo(a). Our results also suggest that the larger the protein is, the better the inhibition is. We are currently testing several deletion mutants of apo(a) to identify critical domains for the inhibitory activity and to decipher the mechanism of inhibition.

Altogether, our results identify apo(a) as an additional component of the lipid metabolism modulating HCV infection.

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Abstract no: 5
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Method comparison of VIDAS® ANTI-HBS TOTAL II with three equivalent assays in the 5–40 IU/L range critical for HBV vaccine status establishment

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Vaccine-induced protection from hepatitis B virus (HBV) infection is correlated with the presence of antibodies against HBs antigen and individuals with a response ≥10 IU/L are considered protected. Each anti-HBs assay detects parts of the polyclonal anti-HBs response, depending on its capture phase design. Criteria important for assay performance are equivalent recognition of HBV subtypes ad/ay and metrological agreement at 10 UI/L cut-off. This later is not easy to achieve and discrepancies between assays were repeatedly reported. Around 10 UI/L, discrepant results can translate into opposite clinical decisions regarding revaccination. We have compared 4 anti-HBsT assays in order to determine the one with the fewest discrepancies in the 0–40 IU/L range relevant for revaccination decision.

99 samples of routine HBV vaccine follow-up were collected from 3 laboratories in Grenoble area (CEA, CHU and Oriade Noviale). The recruitment criterion was a first anti-HBs result between 0 and 40 IU/L from one of the VIDAS, Architect or Cobas assays. In addition to these, all samples were also tested with the Biorad assay to generate 4 anti-HBsT results for each sample. For result interpretation, <10 IU/L was considered negative and ≥10 IU/L was positive. Inter-assay qualitative agreements were defined as follows: total agreement is 4/4 positive or negative results, partial agreement is one discrepant result out of 4 and no agreement is 2 vs 2.

54/99 of anti-HBsT results were in total agreement and 35/99 were characterized by only one discrepant assay out of 4. The discrepancies were the following: 4 for VIDAS with 2 relative false positives and 2 relative false negatives, 7 for Architect with 2 relative false positives and 5 relative false negatives, 11 for Biorad, all relative false negatives, 13 for Cobas with 9 relative false positives and 2 relative false negatives. 10/99 of results were indeterminate and showed no agreement (2 vs 2).

Among 4 assays, VIDAS anti-HBs Total II had the fewest discrepancies around the 10 IU/L cut-off owing to excellent analytical characteristics and enabled the most reliable decisions for HBV revaccination.

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Abstract no: 59
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Study of HCV seroprevalence in adult population in the Czech Republic

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Background: Last official HCV seroprevalence survey in the Czech Republic (CZ) was performed by National Institute of Public Health (NIPH) in 2001 with HCV prevalence determined only 0.2%. Nevertheless chronic hepatitis C (VHC) is one of the frequent indication for liver transplantation in CZ (15.6%). According to the official report of NIPH up to 1000 of VHC cases and 82% VHC of all chronic hepatitis are reported annually in CZ (2015). The aim of our work was to determine the seroprevalence of HCV in CZ adult population, HCV viraemia and HCV genotype in HCV RNA positive persons and analyze the results as to the risk factors (i.v. drug users, health care workers) and estimate the number of persons with chronic hepatitis C in CZ.

Materials and methods: The examined group included 3000 adult persons visiting in 02–09/2015 research centres of Hradec Kralove, Brno, Ceske Budejovice, males in 48.83%, females in 51.17%, age 18–90, median age 46 years. Anti-HCV antibodies were examined by 3rd generation test, CMIA (enzymatic immunoassay with chemiluminiscent detection), on Architect i2000, Abbott, with cut off S/CO <1 = negative (nonreactive); 1–2 = borderline reactive; >2 = positive (reactive). Samples with borderline reactivity were confirmed with immunoblot RIBA in NIPH. To determine viraemia all anti-HCV reactive samples were examined by RT–PCR, in HCV RNA positive samples genotypes were determined.

Results: Of 3000 samples 50 were determined anti-HCV positive, seroprevalence of 1.67% (2.39% in males, 0.98% in females). 12 borderline reactive samples were confirmed negative by RIBA.

Overall prevalence of HCV RNA positivity was 0.93%, 1.5% in males and 0.39% in females. Of all examined subjects 47 were i.v. drug users, 51.06% of them were anti-HCV positive, 23.4% HCV RNA positive. 0.88% of anti-HCV positivity was in the rest of examined subjects (non-drug users) with 0.58% of HCV RNA positivity. HCV genotypes were determined: 1a in 25%, 1b in 25%, 3a in 46% (4% nondetermined).

Conclusion: Since 2001 HCV seroprevalence has increased eightfold up to 1.67% with higher prevalence in males and in drug users. Highest HCV seroprevalence and chronic VHC was determined in males in age group of 30–44 years. with the increase of 3a genotype (31.1% vs 46%). Recounted to the total number of inhabitants we can estimate there are more than 140,000 persons with VHC anamnesis and more than 80,000 persons living with chronic hepatitis C in the Czech Republic.

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Abstract no: 7
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The Syrian refugees crisis brings challenges to the health authorities in Europe: Hepatitis A Virus is a case in point

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The ongoing three-year Syrian Civil war has left hundreds of thousands killed or wounded in addition to the displacement of more than 6.5 million Syrians throughout the world. According to the United Nations High Commission on Refugees (UNHCR), 10% of the displaced Syrian refugees are seeking safety in Europe where the majorities are concentrated in Serbia and Germany (57%) compared to 31% in Sweden, Hungary, Austria, Netherlands and Bulgaria, and 12% in the remaining 37 European Countries. This influx of Syrian refugees to Europe presents the European health authorities with a serious challenge as it carries with it the potential of introducing infectious diseases that have been eradicated in the continent. These diseases include poliomyelitis, measles, to mention only a few. In contrast to the vaccine coverage against polio and measles in Europe, the overwhelming majority of European countries do not include HAV vaccine in their immunization calendars. Moreover, the incidence rates of HAV infection in European countries has declined since the mid-1990s to very low levels which put susceptible populations at risk of acquiring HAV infection and therefore the greater likelihood of outbreaks in these countries. Consequently, there is a growing public health concern in high-income countries, like most of the European countries, that many adults remain susceptible to HAV infection and are thus at risk of severe HAV symptoms and may be death. The impact/spread of HAV outbreaks among refugees on the hosting European countries is expected as was the case in other hosting countries such as Lebanon, Jordan and Iraq. We believe that new policies regarding HAV vaccination should be implemented in the European countries. Since Western Europe, where most of the Syrian refugees are concentrated, has consistently shown a very low seroprevalence rate of anti-HAV compared to low seroprevalence rates in Central and Easter Europe, perhaps large-scale immunization programs among children more than one year of age in East European countries and vaccination campaigns targeting high-risk groups in the West-European Countries are practical approaches at the present time. This article sheds the light on an immediate action that has to be taken by the European countries in order to control HAV infection and to prevent any future HAV epidemics in the continent.

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Abstract no: 89
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Performance of the Aptima® HCV Quant Dx Assay on the Panther® System

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Background: The Aptima HCV Quant Dx Assay is a fully automated quantitative assay for use on the Panther® system. It is based on real-time Transcription-Mediated Amplification (TMA) technology. The assay measures and quantifies HCV in serum and plasma samples. It can be used an aid in the diagnosis of HCV infection and in the management of HCV infected patients undergoing HCV antiviral drug therapy.

Methods: The Lower Limit of Detection (LoD) and Lower Limit of Quantitation (LLOQ) of the assay were determined from dilutions of the 2nd HCV WHO International Standard (NIBSC 96/798 genotype 1) and HCV positive clinical specimens diluted in HCV negative human plasma and serum. Probit analysis was performed to generate the 95% predicted detection limits. LLOQ was established for each genotype by diluting clinical specimens and the 2nd HCV WHO International Standard (NIBSC 96/798 genotype 1) in HCV negative human plasma and serum following CLSI EP17-A guidelines. Specificity was determined using 200 fresh and 536 frozen HCV RNA negative clinical specimens including 370 plasma specimens and 366 serum specimens. Linearity for genotypes 1–6 was demonstrated by diluting HCV transcript in buffer at concentrations ranging from 1.36 to 7.36 log IU/mL. Precision was tested using a 10 member panel made by diluting HCV positive clinical specimens or spiking armored RNA into HCV negative plasma and serum. A method comparison was conducted against the Abbott RealTi*m*e HCV Assay using 1058 (872 plasma, 186 serum) clinical specimens from HCV infected patients.

Results: The 95% LoD was dependent on the HCV genotype and was 5.1 IU/mL or lower for serum and 4.8 IU/mL or lower for plasma. The LLOQ for the assay was 10 IU/mL for both serum and plasma. Specificity was 100% with 95% confidence intervals of 99.6–100% for serum and plasma data combined. The assay demonstrated good linearity across the dynamic range for all genotypes. Precision was 0.17 log SD or lower across the range of the assay for both serum and plasma. Aptima HCV Quant Dx assay viral load results for clinical specimens were compared to those obtained using the Abbott RealTi*m*e HCV Assay. A slope of 1.07, an intercept of 0.08 and an R^2 of 0.97 were obtained.

Conclusion: The Aptima HCV Quant Dx Assay is highly sensitive and specific. The assay gave comparable HCV viral load results when compared to the Abbott RealTi*m*e HCV Assay. The performance of the Aptima assay makes it an excellent candidate as an aid in the diagnosis of HCV infection and in the management of HCV infected patients undergoing HCV antiviral drug therapy.

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Abstract no: 93
Presentation at ESCV 2016: Poster 129

Detection of Q80K mutation in HCV NS3 protease gene in Hradec Kralove – Initial experience

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Background: Combination of HCV high speed replication, low accuracy and poor HCV polymerase correction result in formation of highly variable viruses overall called "quasispecies" with a high sequential diversity within different genotypes and subtypes. The impact of mentioned situation is the accumulation of virus variants with mutations (originated in aminoacids substitution) with the different grade of resistance to DAA (directly acting antivirals) in naive patients, so called RAV (resistance associated amino acid variants) [1,2].

Polymorphism Q80K in NS3 region belonging also to RAV occurs most often in patients infected with HCV 1a genotype and is associated with a decreased response to simeprevir therapy. Q80K prevalence in these patients varies in different geographical conditions. The study of Q80K prevalence in European population presents the prevalence of 19.8% in patients with genotype 1a and 0.5% in genotype 1b % [4].

At present the patients infected with HCV genotype 1a are examined for the presence of mutation in Q80K codon prior to simeprevir therapy introduction. Further clinically significant mutations described in codons 36, 43, 122, 138, 155, 156, 158, 168 are reported only in 1–2% of cases [1,3,5].

Materials and methods: Our laboratory of molecular biology introduced the detection of mutations in the gene for protease NS3 in 2015 by means of sequence analysis. The primers design was performed using Custom Primers – OligoPerfect™ Designer software. The method was optimized for HCV genotype 1a.

Results: In the period of September 2015 to May 2016 the total of 45 patients were examined, in 10 of them (22%) Q80K mutation was detected.

Reference

[1] M. Ogishi, H. Yotsuyanagi, T. Tsutsumi, et al., Deconvoluting the composition of low-frequency hepatitis c viral quasispecies: comparison of genotypes and NS3 resistance-associated variants between HCV/HIV coinfected hemophiliacs and HCV monoinfected patients in Japan, PLOS ONE 10 (2015) 1–28.
[2] S. Paolucci, L. Fiorina, B. Mariani, et al., Naturally occurring resistance mutations to inhibitors of HCV NS5A region and NS5B polymerase in DAA treatment-naïve patients, Virol. J. 10 (2013) 355–361.
[3] M. Leggewie, V.B. Greenu, T. Abdelrahman, et al., Natural NS3 resistance polymorphisms occur frequently prior to treatment in HIV-positive patients with acute hepatitis C, AIDS 27 (2013) 2485–2488.
[4] C. Sarrazin, E. Lathouwers, M. Peeters, et al., Prevalence of the hepatitis C virus NS3 polymorphism Q80K in genotype 1 patients in the European region, Antivir. Res. 116 (2015) 10–16.
[5] A. Bae, S.C. Sun, X. Qi, et al., Susceptibility of treatment-naive hepatitis C virus (HCV) clinical isolates to HCV protease inhibitors, Antimicrob. Agents Chemother. 54 (2010) 5288–5297.

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Abstract no: 99
Presentation at ESCV 2016: Poster 130

Hepatitis B virus vaccination status of medical laboratory workers; a multicentre evaluation in Turkey

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Introduction and aim: The frequency of hepatitis B infection among health care workers is reported to be 3–8 times more than the normal population, particularly among workers in emergency service, surgery, intensive care unit and laboratory, who are frequently exposed to the contaminated patient materials such as blood and other body fluids [1]. In this multicentre study, we aimed to determine the rates of hepatitis B vaccination in medical laboratory workers in Turkey and aimed evaluate the precautions to be taken on this special subject.

Materials and methods: Total number of 1359 medical laboratory workers from 28 medical centres representative of different regions of Turkey was included in this study. A questionnaire was designed to gather all the data on the subject planned to apply all the medical laboratory workers.

Results: Total number of 1359 laboratory worker was included in this study, and male to female ratio was 0.74 (578/781). Doctors ($n = 133$), research assistants ($n = 78$), laboratory technicians ($n = 196$), biologists ($n = 750$), students ($n = 24$), cleaning staff and other workers ($n = 161$) were included in the study. We determined that HBV vaccine was applied to the 1118 laboratory workers (82.3%) out of 1359. When anti-HBs titre levels of the vaccinated participants were investigated, 715 (54.5%) of the vaccinated participants stated that they had anti-HBS levels above 10 IU/mL, 116 (8.5%) of them told that their antibody levels were below 10 IU/mL and 502 (36.9%) of them stated that they did not know their anti-HBS titre levels. The results of statistical analysis revealed that vaccination rates and occupation groups were correlated among the laboratory staff ($p < 0.05$). However, there was no significant difference between age groups and the duration in work with the vaccination rate ($p > 0.05$). Anti HBs positivity was not correlated with any of the groups ($p > 0.05$).

Discussion and conclusion: Health care professionals are required to make immunization a professional habit to protect themselves from health care associated infections in addition to implantation of standard infection control procedures [2]. Present

study is the first multicentre study to reflect the HBV vaccination rates among laboratory workers across the entire country. According to the findings obtained from this study, it was understood that approximately one third of the laboratory staff was vulnerable to hepatitis B virus infections, which were protectable. As a result, medical laboratory personnel possess the risk of acquiring hepatitis B infection, so that formation of awareness is necessary by way of education. They should be tested and all staff seronegative staff should be vaccinated. Periodic monitoring for anti-HBs levels is also essential. This assessment is a necessity within the scope of infection control measures, workers'health and safety [3].

Keywords: Hepatitis B virus, Surveys and questionnaires, Laboratory personnel, Vaccination

Reference

[1] World Health Organization. Health Care Worker Safety. AIDE-MEMOIRE for a Strategy to Protect Health Workers from Infection with Bloodborne Viruses.
[2] http://www.who.int/injection_safety/toolbox/en/AM_HCW_Safety_EN.pdf?ua=1/02/02/2015.
[3] Z. Karacaer, I.I. Ozturk, H. Cicek, S. Simsek, G. Duran, L. Gorenek, The level of information about the immunization of health care workers, attitudes and behaviors (Saglık çalışanlarının bağışıklanma ile ilgili bilgi düzeyleri, tutum ve davranışları), TAF Prev. Med. Bull. 14 (5) (2015).

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Abstract no: 110
Presentation at ESCV 2016: Poster 131

Treatment of HIV and acute myeloid leukemia by allogeneic CCR5-d32 blood stem cell transplantation

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The Berlin patient is presumed to be the only person cured from HIV-infection by hematopoietic stem cell transplantation (HSCT) from a homozygous CCR5-d32 donor. Attempts to reproduce cure by HSCT have failed because of either viral rebound or death due to the underlying malignancy. We here report a patient alive, well and negative for proviral DNA 900 days after HSCT.

A 41 year old HIV-infected male patient was diagnosed acute myeloid leukemia (AML, inv16, CBF-MYH11) in 01/2011. Since the diagnosis of HIV-infection in 10/2010 he had been treated with TDF/FTC+ DRV (01/2011 VL 44 cop/mL; $CD4^+$ 474 cells/μl). To avoid interactions with chemotherapy DRV was switched to RAL in 03/2011. He achieved CR of the AML after 1 induction course (ICE) and received a 2nd induction and 3 consolidation courses according to AML-SG 07/04. In 09/2012 AML relapsed and he was treated with A-HAM and a 2nd cycle high-dose cytarabine. While in 2nd CR he received unmodified peripheral blood stem cells from a female 10/10 CCR5-d32 DKMS-donor after conditioning with fludarabine/treosulfan in 02/2013. Before transplant HIV resistance analysis was performed and viral tropism was determined. There were no significant resistance mutations and the coreceptor-usage was predicted as R5-tropic (Sanger sequencing: FPR 44.5%; NGS: 0.14% X4 at 3.5% FPR; geno2pheno). The proviral DNA load was 29400 cop/mL and in the western blot all anticipated bands could be detected. During transplant and until today the patient remained on ART (since 06/2014 ABC/3TC/DTG) and the viral load remained undetectable in plasma and liquor. He had a 2nd relapse of AML in 06/2013 but re-entered molecular remission after a total of 8 courses of 5-azacytidine and 4 donor lymphocyte infusions. Concerning HIV, all collected samples were negative for proviral DNA by conventional and digital droplet PCR* in two different labs, namely PBMCs (06/2014, 01/2015* and 02/2015), rectal biopsy (04/2015) and bone marrow (08/2015*). Western blots from 06/2014 and 02/2015 showed incomplete patterns with fading bands.

Like in the Berlin patient, all tests from the Duesseldorf patient so far suggest that HIV may have been eradicated and that he may be the second individual cured from HIV by allogeneic CCR5-d32 HSCT. Further investigations will be performed before considering the discontinuation of ART.

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Abstract no: 119
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Evaluation of the Aptima HIV-1 Quant Dx Assay using plasma and dried blood spots

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HIV-1 RNA quantitation in plasma, or virus load testing, is the primary method by which the response to antiretroviral therapy is monitored. In the current study, we describe the analytical and clinical evaluation of the Aptima® HIV-1 Quant Dx assay (Aptima) performed on the automated Panther® system. Using HIV-1 subtype B, Aptima had a dynamic range extending from 6.7 to 2.0 $\log_{10}$ copies/mL, the lower limit of 95% detection was <20 copies/mL, and within-laboratory precision at low virus loads ranged from percent coefficient of variation (CV) of 8.13% at 1.7 $\log_{10}$ copies/mL to 3.59% at 3.0 $\log_{10}$ copies/mL. The clinical performance of Aptima was compared to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test v2.0 (CAP/CTM) using 162 EDTA plasma samples collected from patients undergoing HIV-1 monitoring. Overall agreement was 84.0% (136/162) with a kappa statistic of 0.723 (Standard Error 0.047; 95% CI 0.630–0.815) indicating substantial agreement. Using the 86 clinical samples quantifiable by both methods, Passing-Bablok regression revealed a regression line of $Y = 1.069X - 0.346$ [95% CI of the slope (1.003–1.139) and intercept (−0.666 to −0.074)] and Bland–Altman analysis demonstrated a mean difference (Aptima-CAP/CTM) of −0.075 $\log_{10}$ copies/mL (95% limits of agreement of −0.624 to 0.475), consistent with negative bias. Comparison of Aptima testing on paired dried blood spot (DBS) and plasma specimens archived from participants in the Peninsula AIDS Research Cohort Study (PARC) demonstrated an overall agreement of 94.7% (90/95) when 1000 copies/mL was used as threshold. In conclusion, the Aptima HIV-1 Quant Dx assay provides a suitable alternative for HIV-1 monitoring in plasma and DBS.

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Abstract no: 141
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Prospective evaluation of Cepheid Xpert HIV-1 Viral Load assay as a supplemental confirmatory HIV-1 test in the routine clinical laboratory setting

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Background: Confirmation of HIV infection is a multistage procedure – in the case of reactive/indeterminate results of the 4th generation screening immunoassay, serum/plasma samples are tested with a confirmatory antibody assay that differentiates between HIV-1 and HIV-2. Recently it was recommended to resolve nonreactive/indeterminate results of an anti-HIV confirmatory test with a HIV-1 nucleic acid amplification test. The Cepheid Xpert HIV-1 Viral Load (Xpert) is an *in vitro* diagnostic test designed for rapid quantification of HIV-1 in human plasma and prediction of disease prognosis, taking into account the clinical presentation and results from other tests. To the best of our knowledge, to date the performance of the Xpert on human serum samples has not been evaluated. The objective of the present study was to prospectively evaluate whether the Xpert can be used off label as a rapid supplemental assay for the same day confirmation of HIV-1 infection and/or rapid resolution of HIV status in either serum or plasma samples with reactive/indeterminate HIV screening test results and indeterminate/negative HIV confirmatory test results.

Materials and methods: The present study included 67 prospectively collected blood samples from 50 consecutive anti-HIV-1-positive individuals with clearly positive results of the confirmatory Geenius HIV-1/2 assay (Geenius) in the initial specimen and from 17 consecutive individuals with initially unresolved HIV-1 status (reactive/indeterminate screening and negative/indeterminate confirmatory Geenius results), out of whom 15 turned to be HIV negative in the follow-up and 2 had acute HIV-1 infection at the time of initial testing. The presence of HIV-1 RNA in all initial samples (27 serum and 40 plasma samples) was determined using Xpert, following the manufacturer's instructions, and compared to the results obtained with Abbott RealTi*me* HIV-1 (Abbott RT).

Results: The presence of HIV-1 RNA was detected using both Xpert and Abbott RT in initial blood samples of all 50 individuals with clearly positive confirmatory Geenius results in initial specimens and in both individuals with initially unresolved HIV-1 status, who had acute HIV-1 infection at the time of initial testing. All 15 individuals with initially unresolved HIV-1 status, who turned to be HIV negative in the follow-up, tested HIV-1 RNA negative in initial blood samples using both Xpert and Abbott RT. In 17 HIV-1-positive serum samples viral loads ranged from 3.33 to >7 $\log_{10}$ cp/ml (mean 4.66 $\log_{10}$ cp/ml) and from 2.45 to >7 $\log_{10}$ cp/ml (mean 4.37 $\log_{10}$ cp/ml) using Xpert and Abbott RT, respectively, demonstrating a good correlation between the two tests (Pearson $r = 0.98$, $R^2 = 0.93$), with an overall mean difference of 0.29 $\log_{10}$ cp/ml (range −0.1 to 0.87). In 35 HIV-1 positive plasma samples viral loads ranged from 1.75 to 6.53 $\log_{10}$ cp/mL (mean 5.07 $\log_{10}$ cp/ml) using Xpert and from <1.6 to = "" 6 = "" 51 = "" log = "" sub = "" > 10 cp/mL (mean 4.84 $\log_{10}$ cp/ml) using Abbott RT, demonstrating a good correlation between the two tests (Pearson $r = 0.96$, $R^2 = 0.92$), with an overall mean difference of 0.23 $\log_{10}$ cp/ml (range −0.5 to 0.8).

Conclusions: According to results of the present study Xpert can be used as a reliable supplemental molecular test for rapid confirmation of HIV-1 infection and/or resolution of HIV status in either serum or plasma samples with reactive/indeterminate HIV screening test results and indeterminate/negative HIV confirmatory test results.

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Abstract no: 17
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Molecular characterization of HIV-1 in HBV ± HDV/HCV co-infected HIV-1 positive patients in Turkey

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Background and aims: Co-infection with either HBV ± HDV or HCV in HIV-1 positive patients is not very common, but possible since all these viruses share transmission routes and geographical distribution. Interaction between these viruses generally amplifies liver damage, increasing the risk of developing end-stage liver disease and hepatocellular carcinoma. HIV/HCV co-infection is associated with poorer response to antiviral therapy. The objectives of this study were to determine the subtypes and the primary ART resistance mutations of HIV-1 in HBV ± HDV/HCV co-infected HIV-1 positive Turkish patients.

Materials and methods: We have 74 co-infections from eleven different province of Turkey [Gender, M/F *n*; 65/9, Age, median years (range); 39 (17–68), $CD4^+$ T-cell count, median mm^3 (range); 389 (3–1659), HIV-RNA load, median IU/ml (range); 5.62 + E5 (6.9 + E2–6.3 + E6), HIV acquisition route, *n* (%); heterosexual contact; 38 (51), MSM; 30 (41), Bisexual contact; 2 (2.7), Injection drug use; 4 (5.3), Co-infection status, *n* (%); HIV-1 + HBV; 56 (76), HIV-1 + HBV + HDV; 3 (4), HIV-1 + HCV; 15 (20)]. HIV-1 subtypes and CRFs were identified by phylogenetic analysis (neighbor – joining method) via sequencing of HIV-1 *pol* gene (CLC Sequence Viewer v7.5, Qiagen Aarhus A/S, Denmark). HIV-1 ART resistance mutations were analyzed according to criteria by the WHO 2009 list of surveillance drug resistance mutations.

Results: The molecular evidence in this study indicates subtype B (51/74, 69%) and CRFs (17/74, 23%) of HIV-1 are most prevalent subtypes. CRFs of HIV-1, that are described in HBV ± HDV and HCV co-infected patients mainly caused from South-East Asia, East Asia and Central Africa (CRF 01_AE), West Africa, Central Africa and Middle East/North Africa (MENA) (CRF 02_AG), South America (CRF 12_BF) and Spain (CRF 14_BG), respectively. HIV-1 ART resistance mutations were detected in 12/59 (20%) and 3/15 (20%) HBV ± HDV and HCV co-infected patients in HIV-1 positive Turkish patients, respectively. However, genotype D/subtype D1(98%) in HBV and type 1b (93%) in HCV infected patients were predominant genotype.

Conclusions: HIV-1 molecular epidemiology studies in the HBV ± HDV and HCV co-infected patients are important tools for tracking transmission patterns and the spread of CRF and monitoring of CRF subtypes of HIV-1 in globally scale may be important in vaccine development against HIV. However, the high prevalence of HIV-1 ART resistance mutations in such as patients suggested that the resistance testing must be an integral part of the management of HIV-1 infection and the choice of first – line therapy regime should be guided by the results of genotypic resistance in Turkey [1,2].

Reference

[1] M. Sayan, A. Gündüz, G. Ersöz, A. İnan, A. Deveci, G. Özgür, F. Sargın, G. Karagöz, A. İnci, D. İnan, A. Ülçay, I. Karaoğlan, S. Kaya, S.S. Kutlu, K. Süer, A. Çağatay, H. Akalın, Integrase strand transfer inhibitors (INSTIs) resistance mutations in HIV-1 infected Turkish patients, HIV Clin. Trials 17 (3) (2016) 109–113.
[2] S. Akhan, M. Sayan, E. Sargin Altunok, A. Aynioglu, A case report: antiviral triple therapy with telaprevir in a haemodialysed HCV patient in Turkey, Acta Clin. Belg. 70 (6) (2015) 440–441.

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Abstract no: 20
Presentation at ESCV 2016: Poster 135

Genotyping of HPV DNA positive and HPV E6/E7 mRNA negative cervical samples with abnormal cytology

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Background: Human papillomaviruses have been established as a risk factor for invasive carcinoma of the uterine cervix. HPV DNA detection, provides an efficient method of screening. Detection of the HPV E6/E7 oncogene expression emerged as a promising biomarker to determine the risk for the progression to high-grade cervical lesions. In the present study, we aimed to determine the genotypes of HPV DNA positive and mRNA negative samples from our previous study.

Material and methods: HPV mRNA and DNA detection in samples with abnormal cytology were evaluated in our previous study. Cervical specimens were obtained at Hacettepe University Hospital, Department of Obstetrics and Gynaecology via cervical brushes during January–October 2011. Real-time PCR (Heliosis Human Papilloma Virus LC PCR Kit, Metis Biotechnology, Turkey) and NASBA assay (NucliSENS EasyQ HPV v1.1, bioMerieux, France) were performed to detect HPV DNA and E6/E7 mRNA, respectively. In house PCR method was used for genotyping of HPV type 18, 31, 33, 35, 39, 45 and detection of E6/7 gene with PCR master mix (Promega Corporation, Madison, WI, USA), PGMY (Metis Biotechnology, Turkey) and E6/E7 consensus primers (Heliks Biotechnology, Turkey). The primer sequences were controlled in BioEdit 3.0 programme comparing with HPV reference strains. Confirmation of the samples was done by 'Line Probe Assay' (INNO-LiPA HPV Genotyping Extra Amp, Innogenetics, Belgium).

Results: Totally 81 women with abnormal cytology result constituted the previous study group. HPV DNA was identified in 73 samples (90.1%) that comprise HPV-16 in 46 samples (63.1%), HPV other than 16 in 15 samples (20.5%) and mixed HPV infections in 12 samples (16.4%). HPV E6/E7 mRNA expression was observed in 45 samples (55.6%). Towards these results, we tried to determine the genotypes of 28 HPV mRNA negative-type 16 DNA positive samples and 15 HPV DNA other than type 16 positive samples; totally 43 samples were tested. Among the 43 samples, 5(12%) samples were detected positive with E6/E7 consensus primers. Four of them were HPV-16 and 1 was HPV genotype other than 16. PCR with positive controls which belongs to genotypes 18, 31, 33, 39 and 45 were carried out. All positive controls worked except HPV-33. Therefore, a new primer for HPV-33 (TIB MOLBIOL, Berlin, Germany) was designed and used. However, it did not work again, although it was tested in several PCR conditions. Beside this, all 43 samples were negative for genotypes 18, 31, 35, 39 and 45. Therefore, confirmation of the samples was further done by using LiPA. Totally 12 samples were tested by LiPA. Genotypes detected by LiPA were in 8 cases different from that investigated by PCR (6, 11, 16, 43, 53, 62, 81, 89). Two samples were identified as HPV-33 and two others as HPV-39 by LIPA. However, in none of these 4 samples, these HPV genotypes could be detected by PCR. Because of the limited amount of sample material we could not test all samples by LiPA.

Conclusion: As a result, depending on LiPA findings, it seems that a number of samples investigated were detected negative by PCR because they had genotypes other than genotypes 18, 31, 35, 39 and 45. Beside this, LiPA amplifies a 65-bp region, and its sensitivity thus might be higher than our PCR method which amplifies 238-bp and 455-bp long regions. Also the E6/E7 gene region is not as conserved as the L1 gene region therefore it is possible to obtain more negative results with E6/E7 PCR.

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Abstract no: 205
Presentation at ESCV 2016: Poster 136

Performance of the LIAISON® XL murex recHTLV-I/II assay

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Background: There are two types of HTLV: HTLV-I and HTLV-II. HTLV-I is associated with adult T-cell lymphoblastic leukemia and B-cell chronic lymphocytic leukemia and a demyelinating disease called HTLV-I associated myelopathy/Tropical spastic paraparesis (HAM/TSP). It is estimated that 15–20 million people are currently infected with human T-cell lymphotropic virus type 1 (HTLV-1) worldwide. HTLV-II is less common and is associated with neoplasias of the CD8T lymphocytes. Transmission of both HTLV I and II occurs through sexual contact, exposure to blood, transfusion of infected cellular blood components and perinatally, probably by breast feeding.

Aim: In this study, the performance of LIAISON® XL murex recHTLV-I/II assay (DiaSorin, Saluggia, Italy) was compared to that of ARCHITECT rHTLV-I/II (Abbott, Wiesbaden, Germany), used routinely in our laboratory.

Methods: During a 2 week period, all unselected serum samples ($N = 663$) submitted to the laboratory for HTLV testing were examined by LIAISON® XL murex recHTLV-I/II assay. Samples that were discordant were tested by INNO-LIA HTLV I/II Score (Fujirebio

Europe N.V, Gent, Belgium) for confirmation. Sensitivity was evaluated using 49 frozen HTLV-I positive serum specimens (confirmed by Immunoblot INNO-LIA HTLV I/II Score).

Results: Among 663 routine samples, 658 samples were negative with ARCHITECT and LIAISON®. 5 and 3 samples were reactive with ARCHITECT and LIAISON® respectively. The 2 discrepancies samples (weakly reactive with ARCHITECT) were not confirmed by immunoblot. LIAISON® XL and ARCHITECT rHTLV had an overall agreement of 99.7% with 100% negative agreement.

The results are summarized in the following table.

LIAISON® XL murex recHTLV-I/II	ARCHITECT rHTLV-I/II		
	Positive	Negative	Total
Positive	3	0	3
Negative	2	658	660
Total	5	658	663

In addition, all 49 positive HTLV-I samples were detected by these 2 assays.

Conclusion: The HTLV assay performance of LIAISON® and ARCHITECT were equivalent. LIAISON® XL murex recHTLV-I/II assay demonstrated very good specificity and sensitivity. It was appropriate for the large-scale screening of samples for HTLV-1/2 antibodies.

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Abstract no: 210
Presentation at ESCV 2016: Poster 137

The seroprevalence of HBV, HCV and HIV in blood donors

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Objective: Transfusion-transmitted infections, are the most important complications in blood banking. Blood centers in Turkey apply mandatory screening tests for HBsAg, anti-HCV, anti-HIV-1/2 and VDRL/RPR to blood donors. We aimed to evaluate the seroprevalence of HBV, HCV and HIV at healthy volunteer blood donor who applied blood centre of Meram Medical Faculty of Necmettin Erbakan University.

Material and methods: In this study, blood donor screening test results between January 2013–April 2016 in Necmettin Erbakan University Meram Medical Faculty Blood Center have been investigated retrospectively. Of all applicants evaluated in terms of donor suitability and 79.099 healthy donors were screened. Sera of blood donors were analyzed for HBsAg, anti-HCV and anti-HIV-1/2 in the automated device by chemiluminescence microparticle immunoassay principle. Autologous and repeated donations were not included in the study. Results of donors were analyzed and seropositivity rates were determined according to years.

Results: According to the screening test results, the rates of seropositivity for HBsAg, anti-HCV, and, anti-HIV-1/2 were found to be 2.81%, 0.82%, and 0.06% respectively (Table 1).

Conclusion: Transmission of various infectious agents often including viruses to the recipients, is the most common complication of blood transfusion. These agents can cause asymptomatic, acute, chronic and latent infections. Preparation of safe blood for transfusion is done through detailed questioning of donors and screening tests. World Health Organization recommends that screening all donated blood for transfusion-transmitted infections like HBV, HCV, HIV and syphilis should be mandatory. In our study, we determined the prevalence of HBV, HCV and HIV in Konya region with these parameters. The ratios obtained in blood center are consistent with similar studies conducted in Turkey.

Table 1
HBsAg, anti-HCV, and anti-HIV-1/2 seropositivity rates.

Years	Positive HBsAg	Positive anti-HCV	Reactive anti-HIV-1/2
2013	2.3%	0.95%	0.02%
2014	2.9%	0.8%	0.11%
2015	3.05%	0.73%	0.06%
2016	3.2%	0.81%	0.01%
Total	2.81%	0.82%	0.06%

Keywords: Blood donor; HCV; HIV; HBV; Seroprevalence

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Abstract no: 227
Presentation at ESCV 2016: Poster 138

Characterization of Epstein–Barr Virus LMP1 deletion variants by Next-Generation Sequencing in HIV-associated Hodgkin Lymphoma (French ANRS CO16 LYMPHOVIR cohort)

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Background: Among HIV+ patients, Epstein–Barr virus (EBV) is associated with 80–100% of Hodgkin's Lymphoma (HL) cases and the viral oncogenic protein LMP1 (latent membrane protein 1) is regularly expressed in the tumoral Reed-Sternberg cells. Two C-terminal deletion LMP1 variants (del30-LMP1 and del69-LMP1) have been described in animal models to be more tumorigenic than Wild-Type-LMP1 (WT-LMP1). This work aimed to characterize the LMP1 variant frequency with a next generation strategy in HIV+/HL+ patients of the French prospective ANRS CO16 LYMPHOVIR cohort.

Methods: The cohort recruited 82 HIV+ patients with Hodgkin Lymphoma (HIV+/HL+) between 2008 and 2015. Fifty-five whole blood samples (WB), 45 oropharyngeal cells pellets (OC) and 19 tumor biopsies (paraffin-embedded) from these HIV+/HL+ patients were available for analysis. Forty-seven HIV-positive patients without lymphoma (HIV+/HL−) and 14 HIV-negative patients with HL (HIV−/HL+) were recruited as control populations at the Grenoble University Hospital and provided WB and OC samples. After total DNA extraction, the C-terminal region of *LMP1* (344 bp) surrounding the 30 bp and 69 bp deletions was amplified by nested-PCR and sequenced by next-generation sequencing (GS Junior – Roche 454).

Quality trimming and data analysis were performed with the AVA software.

Results: *LMP1* gene was successfully amplified and sequenced in 131 samples from 88 patients (i.e. LMP1 was detected in 35 (63.6%), 24 (51.1%) and 3 (21.4%) of the WB and 23 (51.1%), 35 (74.5%) and 6 (42.9%) of the OC for HIV+/HL+, HIV+/HL− and HIV−/HL+ patients, respectively. The *LMP1* gene from HIV+/HL+ patients could only be amplified in 5 (26.3%) out of 19 tumor biopsies.

The frequency of WT-LMP1 and del30-LMP1 was not different between the 3 groups of patients regardless the sample analysed. The Del69-LMP1 variant was only detected in HIV+ patients. In 21.4% of cases (24 patients), the variant detected in the patient's WB and OC specimens was different.

In 4 out of 5 biopsies of HIV+/HL+ patients, only one LMP1 type was detected (3 WT-LMP1 and 1 del30-LMP1). In the fifth biopsy the del30-LMP1 variant was dominant, represented at 99% with only 1% of WT-LMP1. For only two of these patients, we successfully sequenced LMP1 in all biological compartments (WB, OC and tumor biopsy). One patient presented the same del30-LMP1 variant in the 3 compartments. The second patient harbored only the WT-LMP1 in the tumor biopsy and both WT-LMP1 and del30-LMP1 in the 2 other compartments (60/40% and 50/50% WT-LMP1/del30-LMP1 in WB and OC, respectively).

In 17 out of 131 samples (13%), the NGS technology allowed to detect LMP1 variants with a sensitivity below 15% and as low as 0.5%. Furthermore, in the sequenced LMP1-region, 85 sporadic mutations were observed, 74 of which were non-silent. Among these substitutions, 24 are located in the CTAR2 domain of LMP1.

Conclusion: Further analysis of tumor biopsy are needed (work in progress) but this preliminary study showed the heterogeneity of the LMP1 wild type or LMP1 variants detected with NGS technology without clear differences in the frequency of LMP1 wild type versus LMP1 variant among HL+ or HL− patients.

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Assessment of laboratory performance in the molecular detection of HIV through International EQA Scheme

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Various nucleic acid assays have been developed for diagnostics and therapeutic monitoring of acute human immunodeficiency virus (HIV) infection. Early detection of these acute viral infections is vital and considering that these viruses have high replication rates, early detection upon transmission is crucial. Viral monitoring and early viral load may allow the infection to be halted before clinical symptoms are apparent. Additionally changes in viral load in the patient may indicate the need to modify treatment strategies to prevent further disease progression.

The generation of reliable and reproducible test results is therefore of great importance in clinical settings. The development of international standards has helped considerably in improving test reproducibility within the laboratory, which in turn helps facilitate comparison of results across laboratories. External Quality Assessment (EQA) schemes provide a valuable tool in support of standardization. The design of these schemes allows comparison across multiple laboratories using different molecular assays.

We report the first year's results of recently launched international EQA scheme focused on the nucleic acid detection of HIV. Altogether 22 laboratories from 9 different European countries participated in five HIV scheme rounds organized during 2015-2016. Eleven different commercial assays were reported and most common methods were RealTime HIV-1 assay (Abbott) and COBAS Ampliprep/COBAS Taqman HIV-1 assay (Roche). Method used for pre-testing value determination performed by expert laboratory was Cobas Ampliprep/COBAS TaqMan HIV-1 v2.0 (Roche) in each round. Reported results were statistically evaluated and showed a trend of excellent qualitative performance. All assays used in laboratories indicated a high degree of specificity. EQA scheme is suitable for qualitative and quantitative analyses. Results are scored based on qualitative results, but laboratories report also quantitative results, when applicable, and many laboratories also reported copy number results. Overall precision in quantitative determination has been fairly good. Interestingly, in quantitative results notable differences compared to the pretested values were observed in every round. As high as ten-fold difference in copy number count were observed between participants and as compared to the pretesting result. The results from this first year of the scheme strengthen the importance of external quality assurance programs for HIV nucleic acid quantification to ensure the quality of testing and diagnostics.

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Analysis of the mRNA expression of DNA damage response genes in HIV infected patients

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Though combined antiretroviral therapy (cART) in HIV-1 positive patients allows a massive suppression of viral replication, many aspects of persistence and pathogenesis of infection are still unknown. It has been reported that virus-induced cell killing is triggered by viral integration. Infection by wild-type HIV-1, but not an integrase-deficient mutant, induced the death of activated primary CD4 lymphocytes. Similarly, integrase inhibitors abolished HIV-1-induced cell killing both in cell culture and in $CD4^+$ T cells from acutely infected subjects. The mechanism of killing during viral integration involved the activation of DNA-dependent protein kinase (DNA-PK), a central integrator of the DNA damage response, which caused phosphorylation of p53 and histone H2AX.

The aim of the study was to evaluate if Inhibitor Integrase (INI) containing regimen could affect mRNA expression profile of DNA damage response genes involved, in HIV infected patients.

Forty PBMC samples from HIV+ patients (18 treatment naïve and 22 treated with ART containing INI) and 10 sample from healthy donors (HD) were collected; mRNA levels of FasR, XRCC1, Lig IIIα, Parp-1, DNA PkI, DNA PkII were evaluate using Syber Green Real time PCR (Agilent Technologies). All HIV treated patients had undetectable viremia. Results were normalized using housekeeping genes beta-actin (ΔCT). The fold-difference of expression levels between three groups were measured comparing ΔCT val-

ues. Differences between the groups were analyzed for statistical significance using *T*-test.

A significantly higher expression of mRNA levels of XRCC1, DNA PkI and FasR was detected in HIV infected individuals than in HD (XRCC1: ΔCT naïve = 13.7; ΔCT INI-cART = 15.2 and ΔCT HD = −5.5; $p < 0.05$. DNA Pk1: ΔCT naïve = 9.7; ΔCT INI-cART = 14.3 and ΔCT HD = −2.2; $p < 0.05$; FasR: ΔCT naïve = 14.5; ΔCT INI-cART = 12.3 and ΔCT HD = −0.4; $p < 0.05$).

No significant differences in expression of DNA Pk II, Lig IIIα and Parp-1 mRNA levels between treatment naïve patients, ART containing INI treated patients and healthy donors were detected (DNA Pk II: ΔCT naïve = 6.7; ΔCT INI-cART = 5.3; ΔCT HD = 11.14; Lig IIIα: ΔCT naïve = 8.1; ΔCT INI-cART = 8.44; ΔCT HD = 13.08; Parp-1: ΔCT naïve = 4.6; ΔCT INI-cART = 5.6; ΔCT HD 8.1; $p > 0.05$).

The expression levels of some DNA damage genes (XRCC1, FasR and DNA PKI) are higher in HIV+ patients than in healthy donors. No difference of DNA PK II, Parp-1 and Lig III alpha mRNA expression levels were observed between HIV+ patients and HD. Interestingly, no significant difference between naïve and INI-cART treated patients was observed. This data suggests that a cellular damage persist despite suppression of viral replication.

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Molecular studies on HSV: Replication rate, infection capacity and progeny

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Introduction: In the last years genital herpes has emerged as one of the most prevalent sexually transmitted infections. Herpes simplex virus (HSV) is the most common cause of genital ulcer disease, with infections caused by both sub-types HSV-1 and HSV-2. A better understanding of the virus replication cycle is relevant to the pathogenesis of human diseases and is essential for the development of antiviral chemotherapy.

Objectives: We aimed to shed some light on the HSV-1 and HSV-2 infectious cycle, namely their capacity of infection, replication rate and progeny, in three distinct cell lines (Vero, Vero E6 and HeLa229). We also aimed to evaluate whether the concentration of virus has any influence on the degree of the infection.

Methodology: Preliminary assays were performed in order to understand which cellular concentration, viral load, nutrients' availability and inoculation *modus operandi* (centrifugation *versus* agitation) best mimic the HSV infection. Confluent cell monolayers were infected with two HSV-2 and two HSV-1 at MOIs of 1:10, 1:1, 10:1 and 100:1. Inoculations were performed in parallel in two 24-well plates, one for quantitative real-time PCR (kPCR) and one for immunofluorescence assays, which were incubated for 30 h at 37 °C and 5% CO_2. At different times-points of infection (6, 12, 18, 24 and 30 h p.i.), the wells were scratched for kPCR and the slides were stained with monoclonal antibodies. For kPCR assays, appropriate standard curves were generated by serial diluting plasmids cloned with HSV-1 and HSV-2 single copy genes.

Results and conclusions: Preliminary assays showed that, regardless of the viral load, it takes approximately 23 h for the virus to complete the infectious cycle taking into account that no replication is observed after this time point. Considering the comparison between the two inoculation procedures (centrifugation *versus* agitation), we only observed relevant differences for lower viral loads, with centrifugation yielding more viral progeny. More specific data regarding both the HSV-1 and HSV-2 replication capacity for different MOIs are currently under evaluation.

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Abstract no: 318
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Genetic diversity and drug resistance profiles of human immunodeficiency virus type 1 (HIV-1) strains infecting pregnant women in the Greater Lisbon

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According to the UNAIDS (The Joint United Nations Programme on HIV/AIDS), human immunodeficiency virus (HIV) infected 36.9 million people at the end of 2014, of whom 2.6 million were children under 15 years of age. Vertical transmission is the main cause of infection in children and while the associated risk has decreased dramatically with the introduction of highly active antiretroviral therapy (HAART), this transmission continues to occur. Although it has been the subject of significant progress in recent years, the access of pregnant women to therapy still remains difficult in certain regions. Moreover, in some cases, the presence of viral mutations associated with resistance is responsible for the failure of the implemented prophylactic regimens and may consequently lead to transmission of resistant viruses to newborns.

This study included a group of 34 multiparous women infected with HIV-1, from whom a sample of peripheral blood was collected within two days after delivery, between 1999 and 2008. The great majority of the women had followed therapeutic regimens for prevention of vertical transmission of HIV-1 during pregnancy. The proviral DNA of 70 samples analyzed was extracted and purified from peripheral blood mononuclear cells, being the amplification of the protease coding region carried out by double nested PCR. After nucleotide sequencing, the genetic characterization of the viral strains by manual phylogenetic analysis was performed, along with the characterization of resistance-associated mutations, as well as other genetic polymorphisms, using the HIVdb program (available at http://hivdb.stanford.edu/).

The study revealed a high genetic diversity of HIV-1 within this population, with predominance of G (47.8%), C (14.9%), and B (11.9%) subtypes, and also a high prevalence of unique recombinant forms (16.4%). Non-B subtypes were responsible for the infection in all women of African origin, and the B subtype was only found in Portuguese women. Additionally, African women were the only infected with subtype C. Considering the mutations associated with resistance to protease inhibitors (PIs), two major mutations (D30N and M46I) and seven minor mutations (L10I, L10V, L33F, G48E, A71T, A71V, and T74S) were identified, in 19 of the sequences studied. Of these, 16 were classified as non-B subtypes, but no statistically significant association was found. Furthermore, most of these mutations were detected in women whose prophylactic regimens included PIs, which may have led to their selection. The remaining genetic polymorphisms, not associated with anti-

retroviral resistance, were mainly detected in non-B subtypes, with some being classified as subtype "signatures".

The identification of HIV-1 mutations associated with resistance to PIs in women whose prophylactic regimens during pregnancy included this class of drugs may have implications for the prevention of vertical transmission, stressing the importance of mutation surveillance.

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Substantial increase of newly reported cases of HIV related to MSM in the Czech Republic

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The Czech Republic (10.5 millions inhabitants) belongs to countries with low prevalence of HIV. Since the year 1985 until 2015, 2620 HIV cases have been reported. Considerable continuous increase of newly reported cases of HIV begun in the year 2002 (50 cases) and reached 266 cases in the year 2015. The predominating mode of transmission was sexual with prevailing homosexual transmission. Proportion of HIV infected men who have sex with men (MSM) continuously rose and reached 78% in 2015 while that of heterosexual transmission decreased to 17%. Proportion of HIV infected intravenous drug users was steadily low not exceeding 5%. Over a long period, about half of the newly diagnosed HIV cases had place of residence in Prague. The foreigners with long-term stay in the Czech Republic comprise 20–30% of newly reported HIV cases. About 15% of new cases were detected in acute stage, and another 15% were late presenters. Annual numbers of new AIDS cases varied in the range 18–32 during the last decade. In about 2/3 of these cases HIV infection was newly detected only at this stage. HIV infection in the Czech Republic, in spite of low overall prevalence, shows an increasing trend due to growing incidence in the MSM. High-risk behaviour of MSM is illustrated by steeply increasing occurrence of early syphilis and lymphogranuloma venereum. Currently, the proportion of MSM in newly diagnosed HIV cases in the Czech Republic is among the highest in Europe.

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Genital herpes in a STD outpatient clinic in Lisbon

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Introduction: Genital Herpes is the major cause of genito-ulcerative disease affecting a considerable number of individuals worldwide and is a chronic, life-long viral infection caused by both HSV1 or HSV2. Most cases of recurrent genital herpes are caused by HSV2, being the leading cause of genital ulcer disease in developing countries, but the proportion of anogenital herpetic infections attributed to HSV1 are increasing, especially in young women and MSM. The prevalence in the general population ranges from 10% to 80% and depends on socio-economic factors. Seropositivity rates are higher in women than in men and increase with age. Reactivation and subclinical shedding is more frequent in genital infections caused by HSV2 than by HSV1, which reaffirm the importance of laboratory confirmation of clinical diagnosis.

Aims: Retrospective study of the role of HSV1 and HSV2 infections in genital ulcerations from a population of a Sexually Transmitted Diseases Outpatient Clinic, according to epidemiological, laboratory and clinical data.

Methodology: 56 ulcer genital/urethral swabs from patients suspected of HSV infection were sent to the National Institute of Health (NIH), in Lisbon, between April 2015–April 2016. HSV1 and HSV2 were determined by a quantitative commercial real-time PCR kit, which targets a fragment of 162 bp of a region located in the US7 gene for HSV1 and a fragment of 177 bp of a region located in the US2 gene for HSV2. The 56 swabs were also inoculated in Vero cell cultures for determination of cytopathic effect.

Results: HSV infection in genital/urethral swabs were detected in 30 (53.6%) of 56 samples. The symptoms of the positive cases were genital ulcerations in vulva or penis and/or perineum and the clinical diagnosis was genital herpes infection. In 7 of the 30 positive cases (23.3%) HSV1 DNA was detected (2 man and 5 women with age ranges between 17 and 27 years old); and in 23 of the 30 positive cases (76.7%) HSV2 DNA was detected (18 man and 5 women, with age ranges between 17 and 62 years old).

Five of the 7 HSV1 positive cases were primoinfections (71.4%) and in the 23 HSV2 positive cases, 3 (13.0%) were primoinfections and 8 (34.8%) were the first ulcer episode but not primoinfections.

HSV DNA viral load values varied between 21848–87474493 cop/ml in HSV1 cases and between 1177–31160846336 cop/ml in HSV2 cases. We did not find direct correlation between viral load and primary vs recurrent infection although the higher viral load was found in HSV2 first episode cases. Cytopathic effect was observed in all positive PCR cases. All positive cases were treated with valacyclovir and resolved after treatment.

Comments: To identify HSV genital infections is important for the specific treatment, for preventing the transmission of HSV to partners, and to prevent the risk of acquiring and transmitting HIV. In our study 53.6% cases were positive for HSV genital infection; because of social, demographic and migratory tendency, the population at risk for STI continues to grow and experience an increased burden of disease. We also observed in this population an increasing proportion of HSV1 genital primoinfection, which is in accordance to the literature. In the present study we confirmed the usefulness of real-time PCR for HSV DNA detection in genital ulcerations. Concerning the correlation of viral load with subtype, the differences should be further evaluated with an increase number of clinical cases.

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Abstract no: 33
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Vaginal intraepithelial neoplasia induced by unusual papillomavirus subtype associated with high load of human herpes virus 6

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Introduction: Newly developed molecular technics has allowed the description of hundreds human papillomavirus (HPV) subtypes. These viruses are linked to mucosal and cutaneous lesions and cause 4.8% of cancer worldwide. After integration in basal squamous cells, HPV remains non-activated until production of oncoproteins. The main HPV-induced cancers (cervix, penis, vulva, vagina, anus and pharynx) are caused by different HPV subtypes justifying an orientated HPV subtyping to detect High-risk (mainly HPV-16 and 18) or Low risk HPVs. We report here an atypical case of Vaginal Intra-epithelial Neoplasia (VaIN) linked to HHV_6 and HPV_4 co-infection.

Case report: In 2008, a 54 years-old woman presented during a systematic cervical smear a Low grade Squamous Intraepithelial Lesion (SIL). HPV screening of this sample remained negative using routine technics which screen 16 of the most frequent HPV subtypes (CLART HPV, Genomica, Spain). This patient was put under surveillance to assess the evolution of the lesions. Four years later, the lesions had developed to High grade – SIL (Cervical Intra epithelial Neoplasia grade 2) and metrorrhagia shad appeared. After a non-contributory colposcopy, the suspect area of the cervix was resected and multiple Iodo-negative maculas in the vagina were biopsied analysed and classified as VaIN grade 2 by pathologists. All samples taken during the resection remained HPV-negative using the routine screening test. A wide screening for *papillomaviridae* and *polyomaviridae* was done on all samples to explain the discordance between clinical signs and HPV-negative screening test. Twelve *polyomaviridae* and 101 HPV subtypes were tested but only HPV_4 was found with a significant viral load. All *Herspesviridae* were also screened and a very high load of the HHV_6 subtype A was found in the samples. So the HHV_6 and HPV_4 co-infection was hold liable for the VaIN. Six months later, the remaining lesions were removed by laser techniques leading to the patient's recovery. The patient immunity was analysed (complete blood count, protein electrophoresis, immunoglobulins dosage) but showed no abnormalities.

Discussion: HPV_4 is mostly associated to sun exposed skin cancer like basal cell carcinoma and most of the patients showing these types of non-melanoma skin cancer are immunosuppressed patients. After all, our patient did not present a systemic immunodepression. However, a local immunosuppression could be linked to this clinical emergence of the neoplasia. Indeed, very high local concentrations of HHV6, which target lymphocytes T helpers, may induce severe and local immunosuppression which enhances the oncogenic virus replication. *Papillomaviridae* represent a heterogeneous virus family, with more than 200 different subtypes and it is assumed that most of them remain to be discovered. This case highlights the need for a global approach without a priori in the field of virus-inducted cancers. New technologies like deep sequencing, Whole-Genome Sequencing or multiplex syndromic approach could bring very precious data and help elucidate numerous complicated cases as the one we described here.

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Abstract no: 34
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Inflammatory cytokine and gene expression patterns correlate with etiologic agent causing pneumonia among patients with advanced HIV in Medellin, Colombia

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Objectives: A role for the magnitude of the inflammatory response in clinical course and outcomes has been suggested in patients with HIV. During acute pneumonia, migrating neutrophils and alveolar macrophages secrete a myriad of chemotactic factors and pro-inflammatory cytokines that include reactive oxygen species, IL-8, IL-2β, IL-6, IL-17, TNFα among others. We sought to determine the inflammatory cytokine and gene expression signatures associated with the pathogen (fungal, tuberculosis and bacterial) among patients with advanced HIV, presenting with pneumonia in Medellin, Colombia.

Methods: Patients admitted with pneumonia were enrolled in the study. After informed consent, demographic, clinical and laboratory information was collected. Individuals underwent bronchoscopy and Bronchoalveolar Lavage (BAL) obtained when deemed necessary by the clinical caregiver team. BALs were spun down and supernatant used for multiplex cytokine/chemokine bead arrays, measuring the concentrations of 19 cytokines and chemokines. We adapted RNA-Seq techniques to measure gene expression in BAL. We performed univariate, multivariate and Principal Component Analysis (PCA) to identify proinflammatory profiles that were correlated with fungal, tuberculosis and bacterial pneumonia and we analysed the differentially expressed proinflammatory genes associated with the TB or Fungal infections.

Results: Among 57 patients with HIV, The most frequent pathogens identified by traditional methods were: *M. tuberculosis* (40.4%), Fungi (*Pneumocystis jirovecii, Histoplasma, and Cryptococcus*) (42.2%), Bacteria (8.8%). Using PCA analysis we identified distinct patterns of BAL cytokines associated with fungal infections and *Mycobacterium tuberculosis* infection. We identified genes that are over expressed among patients with TB as the causative agent of pneumonia.

Conclusion: Distinct cytokine/chemokine and inflammatory gene expression patterns are associated with the etiology of pneumonia among HIV infected individuals. These cytokine/chemokine and inflammatory genes may be utilized as biomarkers for etiological diagnosis. In addition, identifying the insight into the inflammatory pathways associated with each pathogen may provide targets for adjunct anti-inflammatory therapy to decrease lung injury.

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Abstract no: 347
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The Alere HIV Combo point-of-care test; Useful in clinical practice?

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Introduction: The Alere® HIV-1/2 Ag/Ab Combo point-of-care-test (POCT) is a commercially available 4th generation point-of-care test for the diagnosis of HIV infection, which allows for detection of acute infection. We evaluated the sensitivity in patients with acute and chronic HIV-1 infection using 95 samples.

Methods: A validation of a point-of-care test using 95 serum samples collected in 2008–2016 and that were stored at −80 °C. Twenty-four samples were only p24 positive; 49 samples were both antibody and p24 positive; 17 samples were only antibody positive and 5 samples were HIV negative. The majority of the samples came from patients attending an outpatient clinic for sexually transmitted diseases in Rotterdam, The Netherlands.

Results: The 90 HIV-1 positive samples were confirmed by the LiasonXL Ag/ab combo assay or the Abbott Architect and Western Blot (in case of antibody positivity), and by PCR or the VIDAS quantitative HIV p24 assay in case of p24 positivity.

The overall sensitivity for diagnosing HIV infection of the Alere test was 91% (82/90) and the specificity was 100% (5/5). Considering the p24 only positive samples, the sensitivity of the Alere test was 63% (15/24). When both antibody and antigen testing were positive, the sensitivity for picking up p24 with the Alere was 22% (11/49), but the antibody component was positive in all samples (49/49).

Conclusion: In a laboratory setting, the Alere® test has an overall sensitivity of 91% to pick up any type of HIV-1 infection. The sensitivity for the diagnosis of acute infection lies between 22% and 63%. We conclude that the test has improved detection of acute infection compared to previous 4G POCT. It performs relatively well in detecting early acute HIV patients and may be beneficial as an initial screening in patients with a recent exposure to HIV.

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Abstract no: 60
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Prevalence of human papilloma virus in HIV-positive patients: A preliminary study

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Objectives: Human papilloma virus (HPV) is an oncogenic virus and some types are the main cause of cervical cancer. HPV types are divided into three main groups on the basis of their epidemiological association with cervical and penile cancer. Fifteen HPV types were classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); three were classified as probable high-risk types (26, 53, and 66); and 11 were classified as low-risk types (6, 11, 40, 42, 43, 44, 53, 54, 61, 70, and 72) [1,2]. Data on HPV prevalence among HIV-infected people in Turkey is limited. In this study, we aimed to investigate HPV prevalence in HIV-positive patients without symptoms of any sexually transmitted diseases.

Table 1
Human papillomavirus types in HIV-positive patients.

HPV type	Infections with one HPV type		HPV types	Infections with multiple HPV types	
	Female (*n*)	Male (*n*)		Female (*n*)	Male (*n*)
6[c]		1	16[a] and 59[a]		1
16[a]	1	2	16[a] and others (39[a], 53[b], 68[a])		1
31[a]	1	1	16[a] and others (31[a], 40[c], 59[a], 66[b])		1
35[a]		1	18[a] and others (42[c], 66[b])		1
42[c]		1	18[a] and others (6[c], 35[a], 40[c], 42[c], 53[b], 66[b], 68[a])		1
45[a]		1	42[c] and 44[c]	1	
54[c]	1	2	44[c] and 53[b]	1	
56[a]		1	51[a] and others (11[c], 53[b], 68[a])		1
73[a]		1	52[c] and others (11[c], 40[c], 54[c])		1
			61[c] and 66[b]		1
			68[a] and others (11[c], 53[b])		1
			68[a] and others (6[c], 42[c])		1

[a] High risk types.
[b] Probable high risk types.
[c] Low-risk types.

Methods: Between September 2015 and April 2016, vaginal and urethral swab specimens were self-collected from 80 [71 (88.8%) male, 9 (11.2%) female] HIV positive patients. The age range of patients is between 20 to 69 years (median: 34 years). Nucleic acid was extracted (Ribospin vRD viral RNA/DNA Extraction Kit, GeneAll, Seegene, South Korea) according to the manufacturer's protocol. Amplification of nucleic acid was performed using DPO primers and Anyplex II HPV28 Detection kit (Seegene, South Korea). PCR products were detected by real-time PCR on BioRad.

Results: Of the 80 patients, 26 (32.5%) were positive and 54 (67.5%) were negative for HPV. Human papilloma virus was detected in 5 (5/9, 55.5%) female patients and 21 (21/71, 29.6%) male patients. More than one human papillomavirus serotypes were detected in 12 out of 26 positive patients. Overall, 21 different serotypes were detected (Table 1). The most common HPV types in patients were 16, 42, 53, 54 and 68. Of the 14 patients infected with one HPV type, nine were high-risk and five were low-risk types. Most of the patients with multiple HPV types were infected with high-risk HPV types. Overall, 18 (69.2%) patients were found to be infected with high-risk HPV types. HPV positive patients were referred to the clinic for follow up.

Conclusion: HPV infection seems to be an important sexually transmitted disease in HIV positive patients presenting to our hospital and should be screened for even in asymptomatic patients.

Reference

[1] N. Munoz, F.X. Bosch, S. de Sanjose, et al., Epidemiologic classification of human papillomavirus types associated with cervical cancer, N. Engl. J. Med. 348 (2003) 518–527.
[2] M. Schmitt, B. Dondog, T. Waterboer, et al., Abundance of multiple high-risk human papillomavirus (HPV) infections found in cervical cells analyzed by use of an ultrasensitive HPV genotyping assay, J. Clin. Microbiol. 48 (1) (2010) 143–149.

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Intermittent HIV-1 low level viremia detection (blips) using the Abbott RealTime HIV assay

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Background: The efficacy of highly active antiretroviral therapy (HAART) is mainly monitored by the HIV RNA viral load (VL) in plasma. Baseline low CD4+ and sustained low level VL (LLVL) have a prognostic value for virologic failure. Moreover, persistent LLVL during HAART is of great concern for potential emergence of drug resistance. Conversely, intermittent detectable VL ("blips") are regarded as having low clinical impact and are no indication for switching HAART. However, increased number and amplitude of blips may lead to a rise in the economic costs of monitoring patients. Aims of this study were to evaluate the rate of blips detection using the Abbott RealTime HIV assay and to analyze their clinical impact.

Methods: VL results from the VIH unit of the Hospital Universitari de Bellvitge (Barcelona, Spain) during the period 2009–2011 were included in the analysis. A blip episode was defined as a single quantified VL detection of 50–2000 copies/mL preceded and followed by <50 copies/mL samples, obtained from patients that were under HAART therapy and had more than 1 year follow-up. Ethical approval for the study was obtained from the Ethical Committee of the Hospital Universitari de Bellvitge.

Results: During the study period, 1600 patients met the inclusion criteria that accounted for a total number of samples of 13123. Median age of the study population was 43 (range 17–86) and 77.1% were males. A total number of 142 blips were identified in the study population: 1.08% of all samples tested, and an 8.88% of the patients. The blip rate remained stable during the three year study period: 1.12, 0.85 and 1.29 respectively for the percentage of all samples tested and 3.42, 2.44 and 3.64 for the annual percentage of all patients. The median VL of blips was 72.5 copies/mL (range 50–1849) and an 83.8% of the detected blips showed a VL below 200 copies/mL (range 78.9–88.2%). Only 23 blip episodes showed rebound above 200 copies/mL and thus, required additional diagnostic testings.

Conclusions: The rate of blips, measured using the Abbott Real Time HIV assay, was very low and remained stable during the study period. The number of episodes requiring additional testing did not have a relevant impact on the economic costs of HIV viremia detection.

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Comparison of genotyping tropism test in paired HIV-1 plasma RNA and proviral DNA from Portuguese patients

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Background: The Human Immunodeficiency Virus type I (HIV-I) infects the host cell, through a CD4 receptor that binds to the gp120 glycoprotein and that requires contact with secondary coreceptors (chemokine receptors), CCR5 (R5) and/or CXCR4 (X4). The viral tropism for HIV-1 is defined by the type of coreceptor used to infect cell: R5, X4 or dual tropism.

Maraviroc (MVC) was the first R5 antagonist approved for the treatment of patients. Viral coreceptor tropism determination is mandatory when the use of CCR5 antagonists is considered. In this context, in case a therapy change is necessary at undetectable plasma viral load, tropism testing may need to be done on either the proviral DNA or the latest plasma sample with sufficient viral RNA; however, the experience with proviral DNA is still limited.

The aim of this study was to analyze the degree of genotyping tropism test concordance between paired plasma HIV-1 RNA and proviral DNA samples from Portuguese patients.

Materials and methods: In this study, we aimed to evaluating the genotypically inferred tropism in paired plasma HIV-1 RNA and proviral DNA samples from 244 HIV-1 Portuguese infected patients.

HIV-1 genotyping tropism test used was an *in house* test. Viral RNA was extracted from 1.0 ml of plasma using a commercial platform (NucliSENS easyMag, bioMérieux). Proviral DNA was extracted using ZR Genomic DNA column kit. Viral RNA was amplified by RT-PCR and proviral DNA by a PCR in the *env* gene. Nested-PCR was performed in both cases to amplify the V3 region of the *env* gene. Each PCR product was sequenced in triplicate. Sequences were analyzed using the ChromasPro software. Tropism was predicted using Geno2Pheno with a false positive rate (FPR) cutoff of 15% for the plasma RNA and 20% for the proviral DNA.

Results: From the 244 samples studied we obtained an amplification rate of 71.3% (174 samples). From these 174 samples, we were able to amplify both plasma RNA and proviral DNA in paired samples from 58 patients (33.4%). In this group, plasma viral RNA and proviral DNA tropism test were concordant in 91.4%. 64.2% of them were R5 and 35.9% X4 in both samples. Only 5 patients had discordant tropism between RNA and proviral DNA. X4 was mostly found in proviral DNA (3/5) and R5 mostly found in plasma RNA (3/5). In 116 patients (66.7%) of the cases, we were only able to detect tropism either in plasma RNA or proviral DNA. 82 were detected only in the proviral DNA being 36, X4 and 46, R5. 37 were detected only in plasma RNA being 15, X4 and 22, R5. In 19 samples with viral load <20 RNA copies/ml we found 12 samples R5 and 7 samples X4 in the proviral DNA.

Conclusions: This study showed a good concordance of the tropism test between plasma RNA and proviral DNA (91.4%) in paired samples as found by others. It seems that the determination of coreceptor can be done either in plasma RNA or in proviral DNA. This test seems to be useful at any stage of the disease. Among discordant samples, the presence of X4 is mainly found in proviral DNA, but when the patients were suppressed, R5 is more frequent in the proviral DNA than X4. This reinforce that prediction of viral tropism using PBMC DNA is feasible, mainly for plasma suppressed patients. Further studies are needed to determine the importance of tropism testing in both compartments, plasma RNA and proviral DNA.

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Case report: Unexpected cause of respiratory failure 3 days after heart transplantation

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Respiratory syncytial virus is an RNA virus belonging to the Paramyxoviridae and it is mostly found in young children. This virus can also cause morbidity and mortality in immunocompromised adults. Respiratory virus infection (RSV) is an important complication in solid organ transplant patients but the longitudinal monitoring of these infections has not been extensively studied. Little has been described in literature regarding RSV pneumonia in adult heart transplant patients.

Here we report an interesting case of a 56 year old female with a history of non-ischemic cardiomyopathy starting in 2011. On January the 3rd of 2015 she successfully underwent a heart transplantation. Although there were no signs of respiratory disease at the time of hospitalization she showed respiratory insufficiency three days post-transplantation.

In the microbiology lab each respiratory sample is cultured and when indicated screened for a panel of 22 targets detected in 8 in-house RT-PCR multiplexes. This molecular panel covers the most important pathogens of viral respiratory infections and atypical bacterial pneumonia. The first respiratory sample of this patient was a bronchial aspirate taken three days post transplantation. The bacterial culture was negative but the sample tested positive for RSV-A with a high viral load (Ct value of 23). Follow up samples 15 days and 35 days post-surgery were still RSV positive although with decreasing viral load (Ct value of 25 and 28 respectively). Culture of respiratory samples showed the presence of *Staphylococcus aureus* only 10 days after surgery so RSV is most probably the primary cause of the respiratory disease. RSV was still detectable 1 month after transplantation which might be explained by the immunosuppressive treatment of the patient. The heart transplantation was performed during the RSV season. Some days before the surgery the lady had taken care of her young grandchildren so there indeed was a potential risk of community-acquired transmission.

Conclusion: Without testing for viral pathogens no accurate diagnosis for the respiratory failure of this patient could have been made. Since screening of adult patients for viral pathogens is not common practice at the IC-unit, this case illustrates the added value of molecular screening when signs of respiratory failure arise in adult immunocompromised patients.

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Abstract no: 148
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Respiratory viruses in the intensive care unit: More frequent than expected

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In the Laboratory of Microbiology of the OLV Hospital in Aalst respiratory samples (n = 3500/year), received from multiple hospitals spread all over Flanders, are analysed on a daily basis by in house multiplex real time PCR for a panel of viral and bacterial pathogens. The panel includes adenovirus, bocavirus, human metapneumovirus (hMPV), respiratory syncytial virus (RSV), parainfluenzavirus (PIV) 1, 2, 3 and 4, Influenza virus A and B, enterovirus, rhinovirus, coronaviruses, *Bordetella pertussis & parapertussis, Mycoplasma pneumoniae* and *Chlamydia pneumoniae*.

Before 2014, the majority of samples originated from children. The severe influenza epidemic in the winter season 2014–2015 made clinicians aware that viral infections in adults are not that innocent at all. Moreover, in the "Influenza season", not only Influenza circulated but also other viruses were cause of severe disease. Correct identification of the pathogen is indispensable to administer or withhold therapy. As a consequence, the request for the real time PCR respiratory panel on samples from adult hospitalized patients increased.

In order to calculate the frequency of these pathogens in adult critically ill patients, a retrospective study was performed for the period September 2014 to May 2016 including patients transferred to the coronary care unit (CCU) and the intensive care unit (ICU) because of respiratory failure.

Respiratory panel results of samples, obtained in the window from 3 days before to 5 days after transfer to the CCU and IC units, were included. From the 126 samples, 44 samples were positive (34.92%) with 41 samples (93.18%) positive for a viral pathogen and 3 samples (6.82%) positive for a bacterial pathogen (1 *M. pneumoniae*, 1 *C. pneumoniae* and 1 *B. parapertussis*). None of the samples were positive for adenovirus or parainfluenzavirus.

As expected, Influenza A virus (n = 14) and Influenza B virus (n = 8) were the most frequent and 1 patient had a co-infection of both viruses. No other co-infection was found. Surprisingly, rhinovirus (n = 8) was found to be the third most frequent viral cause of infection. hMPV and RSV are known to cause severe respiratory problems in infants and RSV infections have also been observed in the immunocompromised host. In our study, not only RSV (n = 5) but also hMPV (n = 7) was found frequently and caused very severe "Influenza-like" disease.

We can conclude that viral infections are a common cause of respiratory problems in the intensive care unit and screening of these patients might be an important clue in diagnosis and correct treatment.

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Multidrug-resistant cytomegalovirus infection in a pediatric stem cell transplantation patient

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Background: Cytomegalovirus (CMV), a member of the *Herpesviridae* family, is characterized by a lifelong latency in the host. Clinical presentations of CMV infection are minimal in immuno-

competent hosts but can lead to life-threatening conditions in immunosuppressed patients.

We report a case of a fatal CMV infection in a 4-month old patient with primary immunodeficiency and consecutive hematopoietic stem cell transplantation.

Case report: A 4-month old girl was referred for fever, failure to thrive and bloody diarrhea. Since she was a child of consanguineous parents, severe immunodeficiency was presumed. CMV was detected in the blood (day −82) with a viral load of 3.77 10E6 copies/mL (in-house real-time PCR) and treatment with ganciclovir was started. As the CMV viral load still increased, therapy was switched to foscarnet (day −33) and patient received CMV-specific immunoglobulins. Antiviral resistance testing (genotyping the UL97 protein kinase, responsible for ganciclovir phosphorylation, and the UL 54 DNA polymerase) in a blood sample on day −19 revealed the presence of a mixed population of M460V-mutant and wild-type virus in the UL97 protein kinase, known to confer resistance to ganciclovir with susceptibility to foscarnet and cidofovir. Despite treatment, patient deteriorated further and developed CMV encephalitis with positivity of CMV PCR on cerebrospinal fluid (day −14), which showed a similar resistance profile as previously analyzed blood samples and nasopharyngeal fluid (NPA) samples.

An allogeneic stem cell transplantation from a haploidentical donor was performed, with both donor and acceptor being CMV IgG positive (day 0). Because of progression of neurologic encephalitic disturbances suggestive of central CMV infection, cidofovir was added empirically to the foscarnet antiviral treatment. Antiviral resistance testing of a blood sample on day +1 showed, in addition to a mixed population of M460V-mutant and wild-type virus at the UL97 protein kinase, also the presence of a mixed population of 981–982 deletion mutant and wild-type virus in the UL 54 DNA polymerase. This is known to confer resistance to ganciclovir, foscarnet and cidofovir. On day +19, only the mixed population of DNA polymerase mutant virus bearing the 981–982 deletion was present in blood. Three days later, the mutant virus totally replaced the wild-type in multiple blood, urine and NPA samples, indicating a generalized multidrug-resistance CMV infection. On day +23, cidofovir was stopped due to severe cytopenia and further increase of viral load. Since respiratory and liver function deteriorated further and the patient developed an uncontrollable sepsis, palliative care was initiated and the patient deceased on day +36.

Conclusions: An immunosuppressed pediatric patient developed a multidrug-resistant CMV disease to currently approved anti-CMV drugs. This case report highlights the importance of rapid drug-resistance monitoring and indicates the urgent need for the development of new anti-CMV drugs.

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Abstract no: 172
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Changing time line of CMV infection in seropositive live donor Liver Transplant recipients: A prospective study from a tertiary care liver center

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Background: To study the incidence and timeline of CMV infections in seropositive live donor liver transplant (LTx) recipients and correlate the risk of infection with pre-transplant CMV immunity.

Methods: A total of 155 consecutive LTx recipients from our Institute, a tertiary care Liver Institute in Delhi, North India were included from March 2010 to April 2012. Patients were not on anti-CMV prophylaxis. Nine cases died during the follow-up, and thus the final analysis included 146 patients. Pre-transplant donor (D) and recipient (R) CMV IgG titers were estimated by Chemiluminescence based immunoassay (Architect, Abbott). Post transplant, follow up was done weekly for 1 month, and then monthly up to one year. Median time of follow up was 299 (±126.7) days. CMV DNA was quantitated in plasma samples using the LightCycler® 480 II Real-Time PCR System (Roche Life Science, US). CMV infection and disease were defined according to the standard criteria. CMV DNA positivity in blood (DNAemia) was considered as the evidence of CMV infection.

Results: Out of 146, 114 (78%) were males, 132 were adults and 14 were pediatric recipients. Pre-Tx 142 (97.3%) were D+R+ and 4(2.7%) were D−R+. Post-Tx CMV infection was seen in 54 (36.9%) recipients. CMV disease was seen in 14 (9.5%) cases. Median CMV viral load was 3.6×10^3 (IQR: 3.4×10^2–4.6×10^6) copies/ml. Significant viremia of ≥500 copies/ml was seen in 45 (29%) cases. CMV infection was higher in pediatric patients 10 (71.4%) than adults 44 (33.3%) (p value = 0.004). Monthly incidence of CMV infection post-Tx was: 32 (59.2%) in 1st, 13 (24%) in 2nd, 5 (9.2%) in 3rd, 2 (3.7%) in 4th, 1 (1.8%) each in 5th & 6th months. Rejection was seen in 30 (19.5%) recipients and was higher when CMV infection was also present 18 (32.7%) as compared to without CMV infection 12 (12%), $p = 0.002$. A total of 113 (77.4%) cases had pre-Tx IgG titers of ≥250 AU/ml. Post-Tx CMV infection in titers <250 AU/mL and ≥250 AU/mL were 42.4% and 34.5%, respectively ($p = 0.99$). There was no difference in the time of occurrence of CMV infection in both the groups (<250 AU/mL vs ≥250 AU/mL).

Conclusions: Early CMV infections were seen mostly within 1st month of post-transplant period in high seropositive population. CMV infection does not correlate with pre-transplant CMV immunity but may contribute in rejection of the transplanted organ.

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BK virus infections in renal transplant recipients

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The BK virus is a member of the polyomavirus family. Infections with BK virus are widespread with a seroprevalence of around 80% in the general population. Following an asymptomatic primary infection, BK virus remains latent in healthy subjects. Reactivation occurs in immunocompromised patients. BK virus is pathogenic mainly among patients who have received a kidney transplant, in whom the virus can cause specific tubulo-interstitial nephritis and even result in graft failure among approximately 20–30% of nephritic cases. The cornerstone of BK virus infection or BK virus-associated nephropathy treatment is to decrease the immunosuppressive regimen, which must then be offset with the risk of rejection. BK Virus Nephropathy (BKVN) occurs in up to 10% of renal transplant recipients (RRT) and can result in graft loss in up to 50% of those affected.

In this study, we retrospectively analyzed the presence of BK virus in plasma and urine samples of patients applied to the Nephrology Clinic of our hospital between 2010–2015. BK virus DNA was determined by real-time PCR using artus BK virus RG PCR kit (Qiagen, Germany) on the Rotor-Gene system (Corbett Research, Australia). The analytical sensitivity of the kit is 0.195 copies/μl according to the user manual.

A total of 243 samples (urine and plasma) from 131 patients (69 male, 62 female), ages ranging from 20 to 72 were enrolled. BK virus DNA was detected in 56 (38.6%) urine samples and in 27 (13.1%) plasma samples. In 19 simultaneously sent urine and plasma sample pairs of 13 patients, BK virus DNA was positive. The minimum and maximum DNA levels of positive urine and plasma samples were as 4–1.4×10^8 copies/ml and 6–5.3×10^4 copies/ml respectively.

In conclusion quantitative viral load monitoring for BK virus (BKV) in urine and plasma samples by real-time PCR is an important tool in the management of polyomavirus associated nephropathy in renal transplant patients.

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The impact of viral respiratory infections in the first year post-transplant period of pediatric hematopoietic stem cell transplant (HSCT) recipients

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Background: Infection caused by respiratory viruses (RV) is a threat for hematopoietic stem cell transplant (HSCT) recipients. RVs in HSCT patients with respiratory syndromes should be strictly monitored in the *pre*-engraftment or early post-*transplantation period and in patients with acute or chronic GVHD.* Due to the high morbidity and mortality rates associated with RVs infections and the lack of directed antiviral therapy for most of these infections, prevention remains the mainstay for reducing their incidence and controlling transmission in HCT recipients. This retrospective study aimed to investigate the incidence and the duration of respiratory episodes caused by viruses in pediatric HSCT recipients.

Material and methods: Patients who underwent allogeneic or autologous HSCT at Pediatric Hematology–Oncology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia from January 2010 to December 2014 were analyzed. Respiratory samples from patients with respiratory syndromes were routinely tested using a panel of RT-PCR and real-rime RT-PCR assays for 12 respiratory viruses within the *first year* post-transplant.

Results: One hundred eighty-six HSCT recipients including 158 (84.9%) allogenic (80 MUD, 56 PMFD, 21 MFD, and 1 sibling) and 28 (15.1%) autologous transplants were evaluated. In 118/186 (63.4%) patients at least one respiratory episode caused by viruses was identified, while 68/186 (36.6%) patients were negative. Among positive patients, 73/118 (61.9%) had a single viral respiratory episode, while 45/118 (38.1%) had multiple episodes (29 with 2 episodes, 8 with 3, 8 with 4 and 1 with 6). In patients with multiple viral episodes, the first episode was observed significantly earlier (median 17.5 days; range 1–349 days) than patients experiencing a single viral episode (median 62 days; range 1–358 days; $p = 0.01$). A total of 192 viral episodes, including 174 (90.6%) single infections and 18 (9.4%) co-infections were observed. Among episodes sustained by a single virus, HRVs were the most prevalent viruses with 54.0% followed by respiratory syncytial virus (13.2%), human coronaviruses (9.2%), human parainfluenza viruses (8.0%), influenza A (6.3%), adenovirus (6.3%), and influenza B (2.9%). Twenty-seven episodes (14.0% of total) of prolonged infections defined as viral shedding ≥30 days were observed. The median duration of viral shedding was 64 days (range 30–159 days). In 18/27 (66.6%) patients, the onset of infection occurred during the induction and before transplant engraftment (<30 days from TX). In these patients, the duration of viral episodes was higher than those observed in the remaining 9 patients, in which the onset

of infections occurred after the engraftment (>30 days from TX) ($p = 0.02$).

Conclusions: Among pediatric HSCT recipients, viral respiratory infections in the post-transplant period are frequent and sometimes prolonged. Preventive measures must be tightened in this population in order to reduce the derived morbidity and mortality.

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Normalizing ELISPOT to quantify human cytomegalovirus (HCMV) and Epstein Barr-virus (EBV) specific T-cell response in kidney transplant recipients

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Background: Herpes virus infection or reactivation are major complications in solid organ transplant recipients. Virus-specific T-cell response is crucial to control infection.

Methods: HCMV and EBV specific $CD4^+$ and $CD8^+$ T-cell response were investigated in 29 kidney transplant recipients by a novel approach of enzyme-linked immunospot assay (ELISPOT). Overlapping 15-mer peptide pools of HCMV proteins immediate early IE-1, IE-2 and phosphoprotein pp65, and of EBV lytic (BZLF1 and BMRF1) and latent (EBNA1, EBNA3a, EBNA3b, EBNA3c, LMP1 and LMP2) proteins were used for stimulation of both $CD4^+$ and $CD8^+$ HCMV-specific and EBV specific T-cells, respectively. Virological and immunological monitoring were performed for one year of follow-up.

Results: As for HCMV infection, 13/19 (68.4%) HCMV seropositive recipients showed levels of HCMV replication <100,000 DNA copies/ml blood and did not required anti-viral treatment, while 6/19 (31.6%) HCMV-seropositive patients were treated since showing ≥100,000 HCMV DNA copies/ml blood. Patients with spontaneous control of infection showed, at 120 days after transplant, levels of HCMV specific $CD4^+$ T-cells significantly higher with respect to patients who needed treatment. HCMV specific T-cell response to single HCMV proteins (pp-65, IE-1, IE-2) was examined: pretransplant number of both $CD4^+$ and $CD8^+$ specific T cells directed against IE-1 showed significantly higher level in patients controlling infection and their level remained significantly higher until 120 days. No difference was shown for pp-65 and IE-2 between the two groups of patients. In addition, 5 HCMV-seronegative recipients receiving organ from HCMV seropositive donor (D+/R−), were examined: 4/5 developed a primary infection within one month from transplantation and required antiviral treatment. HCMV-specific $CD4^+$ T-cells remained significant lower with respect to patients able to control infection until 120 days after transplantation.

As for EBV infection, 3/29 (10.3%) EBV-seropositive patients reaching levels of EBV ≥10,000 DNA copies/ml blood did not showed EBV-specific T-cell response for the entire period considered. However, EBV-specific T-cell response was detected in only 8/20 (40%) patients examined at 1 year follow-up, regardless of the presence of EBV DNA in blood.

Conclusions: Normalizing ELISPOT may be a simple and useful tool to perform immunological monitoring in solid organ transplant recipients and to detect herpes virus specific response. However, while the importance of HCMV-specific T-cell response to control HCMV infection is evident, further studies are required to better define the role of EBV-specific T-cell response.

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BK polyomavirus-seroreactivity increases with virus replication

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Background: BK polyomavirus (BKPyV) infection causes nephropathy in 1–10% of kidney transplant recipients. This condition results in graft-loss in up to 50% of cases unless immunosuppression is lowered. Specific antiviral treatment is not available. In immunocompetent individuals, BKPyV resides latently in kidney tubular epithelium after primary infection during childhood.

In order to predict which recipients will develop BKPyV nephropathy, we recently analyzed a cohort of kidney donor-recipient pairs prior to transplantation for several immunological and virological parameters. That study showed a strong correlation between the strength of BKPyV-seroreactivity measured in the donor and BKPyV infection and nephropathy in the recipient [1]. We hypothesized that BKPyV-seroreactivity of the donors mirrors the load of infectious virus in the transplanted kidney. To further investigate the relation between BKPyV-seroreactivity and BKPyV-replication, we analyzed the dynamics of BKPyV-seroreactivity in individuals that did or did not experienced a detectable BKPyV infection.

Methods: A group of 101 kidney transplant recipients was analyzed for BKPyV-seroreactivity (VP1-antigen; Luminex immunoassay) and for BKPyV viremia (viral load measured by q-PCR). Serum and blood plasma samples were obtained before transplantation and at 3-month intervals until 18 months after. As controls 87 healthy blood donors were analyzed with a 12-month interval. Descriptive statistics and mixed model analysis was used to analyze the association between measured peak BKPyV-loads and BKPyV-IgG seroreactivity.

Results: At baseline the overall BKPyV-seropositivity was high in both transplant recipients (92%) and blood donors (99%). The mean baseline BKPyV-IgG level in both groups was comparable. In 85% of the kidney recipients, BKPyV viremia was detected at some point during follow-up, with peak viral loads ranging from 10 to 579700 copies/ml, while no viremia was detected in the blood donors. After a year, in the healthy blood donors, the mean level of seroreactivity remained the same ($p = 0.929$). This was also the case among kidney recipients without BKPyV viremia ($p = 0.981$). Among kidney recipients that did develop viremia, however, a statistically significant increase in BKPyV-seroreactivity was observed

($p < 0.001$). Stratified analysis showed that the increase in BKPyV-seroreactivity was correlated with the height of the peak viral load measured after transplantation.

Conclusion: In kidney recipients that experience active BKPyV infection, BKPyV-seroreactivity is correlated with BKPyV viremia and dependent of the peak viral load. Based on our previous findings showing that donor BKPyV-seroreactivity predicts BKPyV infection in recipients [1], we believe our current findings provide a template to understand BKPyV infection in general, where BKPyV-seroreactivity in an individual reflects primary BKPyV replication in the past and the level of latent virus in the kidneys. In that way BKPyV-seroreactivity in kidney donors can predict the amount of latent, potentially infectious virus in the kidney allograft.

Reference

[1] Wunderink, et al., Am. J. Transplant. (2016).

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Frequent HHV6 DNA positivity in children with severe burn injury

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Background: Burn injury harms human immunity at multiple levels including decrease of cell mediated immunity and the cytokine storm. Such conditions are well known trigger of herpesvirus reactivation. Aim of our study is to elucidate the frequency and possible impact of herpesvirus reactivation in children with severe burn injury.

Methods: Enrollment criteria: age under 10, severe burn injury (grade II, >5% BSA), fever (day 3 after onset) and low inflammatory parameters (CRP, PCT, normal WBC).

Enrolled children were tested for HSV, VZV, EBV, CMV and HHV6 DNA in blood by multiplex PCR (Seeplex Meningitis – V1, Seegene) and real-time PCR (CMV HHV6,7,8 R-gene, Biomerieux). Tests for anti HHV6 antibodies and avidity were performed by IIF and EIA (IgM – Anti HHV6 IIFT IgM, Euroimmun, IF-VIDITEST anti-HHV-6 IgG, Vidia and IgG ELISA-VIDITEST anti-HHV-6 IgG, Vidia).

Results: Up to date we have enrolled 17 children. The mean age of affected children was 2 (median 2, range from 1 to 7 years). The most frequently detected virus was HHV6 (10/17), among these twice in combination with CMV and EBV, once in combination with EBV. All of them were HHV6 type B with viral load ranging from 91 to 2410 copies per ml of whole blood (median 323 cp/ml). Only one HHV6 DNA positive child was negative for HHV6 IgG and IgM antibodies (ELISA and IIF). Two children with HHV6 DNA were positive for anti HHV6 IgM antibodies. The remaining 7 were IgM negative. All patients positive for HHV6 IgG by EIA had high-avid antibodies. History of exanthema subitum was known only in one child. No rashes or cytopenias were observed during the hospitalization period.

Conclusion: We have not observed any typical clinical signs previously described for active HHV6 infection in immunocompromised patients in our group. Our patients are of age close to time of HHV6 primary infection. The presence of HHV6 DNA in blood is probably a residue of HHV6 integration to the blood progenitor cells during the primary infection, although none of the children had recent primary infection due to the presence of high-avid antibodies.

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BK polyomavirus infection activates the type I interferon response in human fibroblasts, depending on the cGAS-STING pathway

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BK polyomavirus (BKPyV) associated nephropathy is one of the major causes of renal allograft dysfunction. Immunosuppressive therapy favors viral reactivation and nephropathy can develop due to local inflammation and insufficiency of the antiviral immune response. Recent studies have focused on the BKPyV-specific T cell responses, however how BKPyV is detected and whether it induces an innate immune response remain mostly unknown.

Our aim was to investigate innate responses to BKPyV infection in permissive cells and identify intracellular sensors involved in the recognition of the virus.

Human foreskin fibroblastes (HFF) and human renal proximal epithelial cells (hRPTECs) were infected with BKPyV (Gardner strain), and Chikungunya or IFNa as positive controls. IFNb, MxA, IL1b and IL18 gene expression were measured by RT-qPCR, normalized to GAPDH, and results expressed as fold induction over mock-infected cells. At the protein level, MxA expression was measured using flow cytometry and IFN type I levels were measured using a bioassay (HL116 cells) in the supernatant of infected and control cells. We show that BKPyV infection activates MxA expression and type I IFN in HFF, but not in hRPTECs.

To identify intracellular receptors involved in the sensing of BKPyV during infection of HFF, we transfected cells with different siRNA targeting molecules known to be involved in the recognition of viral nucleic acids (IFI16, cGAS, DNA PK, STING, IRF3). Total RNA was harvested at 3 and 6 dpi. Silencing was assessed by RT-qPCR, normalized to GAPDH and plotted as fold induction over the siSCR condition. We observed a significant inhibition of MxA and IFN type I induction after six days post infection when cGAS, STING and IRF3 were silenced, suggesting the involvement of this sensing pathway in the recognition of BKPyV during viral multiplication in permissive cells.

Moreover, BKPyV DNA replication was measured using in house quantitative PCR with and without IFNα. DNA viral loads after three and six days post infection were significantly lower in cells cultivated with IFN α, compared to untreated cells, showing that IFNα restricts BKPyV replication in HFF and HRPTECs.

Altogether, our results show that BKPyV is restricted by IFN type I. BKPyV infection in hRPTECs does not activate strongly the type I interferon response, when this response is significant in HFF. In HFF, this activation depends on the cGAS-STING-IRF3 pathway.

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Monitoring of cytomegalovirus-specific immunity using the QuantiFERON-CMV assay in hematopoietic cell transplant recipients: Preliminary results

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Introduction: Human Cytomegalovirus (CMV)-associated complications are often associated with high morbidity and mortality in patients submitted to allogeneic hematopoietic stem cell transplant (allo-HSCT) despite the improvement in the clinical management of CMV infection either by viral monitoring or antiviral prophylaxis. The reconstitution of antiviral cellular immunity after allo-HSCT is crucial for the prevention of viral reactivations/infections and associated complications. In fact, antiviral cell-mediated immunity specific to CMV is considered crucial for the control of CMV replication, and therefore, monitoring the interferon γ (IFN-γ) produced by CMV-specific T-cells may be useful as a prognostic marker of CMV infection.

Aim: The aim of this study was to evaluate the levels of CMV-specific IFN-γ produced by CD4+ and CD8+ T-cells in patients submitted to allo-HSCT and evaluate its utility in clinical management of CMV.

Material and methods: We have selected five consecutive allo-HSCT recipients considered of high risk for CMV infection. All patients received stem cells from a HLA mismatched/unrelated and CMV IgG seropositive positive donor; 4 patients were CMV-seropositive (R+) and 1 CMV-seronegative (R−). All patients were monitored for CMV infection using pp65 antigenemia or quantitative real-time PCR. The monitoring of IFN-γ levels produced by CMV specific T-cells were evaluated weekly from day 60 post-transplant in 3 consecutive evaluations using QuantiFERON-CMV® assay.

Results: The QuantiFERON-CMV assay revealed that 3 patients had cellular immunity to CMV and 2 had no detectable immune response (IFN-γ levels <0.2 cut-off). During the follow-up period two patients had CMV reactivation/viremia on the first 7 days of follow-up (both D+/R+): one showed low levels (<1.0) and the other was non-reactive for (<0.2) of CMV-specific IFN-γ. Of the 3 patients who had no CMV reactivation, 1 was non-reactive and 2 presented high levels of CMV-specific IFN-γ. Our preliminary results showed that conditioning regimens might influence the CMV-specific immune response: the 2 patients that underwent reduced intensity regimen (RIC) were able to mount CMV-specific immune response; while of the 3 patients submitted to myeloablative regimen (MA), 2 were non-reactive and 1 showed low levels of CMV-specific IFN-γ becoming non-reactive at the 3rd evaluation.

Conclusion: These are a preliminary results from a prospective study involving allo-HSCT patients from Portugal. The results revealed that patients with high levels of cellular immune response to CMV seems to have a lower risk of developing CMV reactivation than those who do not have a detectable immune response or with low levels of CMV-specific IFN-γ. Our preliminary results also showed that QuantiFERON-CMV test could be an important tool in CMV monitoring after allo-HSCT. However a clinical cut-off for QuantiFERON-CMV should be investigated to distinguish patients with high or low risk for CMV-associated complications.

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HHV-6 chromosomal integration in allogeneic haematopoietic stem cell transplantation

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Objectives: Chromosomal integration of HHV-6 is an interesting biological phenomenon of distinct impact of Ci-HHV-6 carriers. According to the published knowledge, Ci-HHV-6 genome is at least partly transcribed with a pro-inflammatory impact documented recently by increased risk of cardiac angina pectoris. The observed frequency of Ci-HHV-6 in the population of the Czech Republic is about 1%. Therefore we wanted to find out the frequency of Ci-HHV-6 among the allogeneic haematopoietic stem cell transplant (alloHSCT) recipients and its possible impact on the CMV infection.

Methods: Between January 2003 and June 2015, we tested for presence of HHV-6 7857 samples from more than 37,000 whole blood samples send for viral surveillance from 326 children and 652 adults after allogeneic HSCT. At least one sample was tested pre- and one after HSCT after stem cell engraftment and recovery of haematopoiesis was obtained in 839 donor/recipient combinations and so we tested presence of HHV-6 both recipient and donor haematopoiesis. DNA extraction was performed using Qiagen Blood Mini Kits according to the manufacturer's instructions and testing was performed subsequently using RQ-PCR technology for HHV-6, same as for CMV and EBV in the samples. In the recipients, Ci-HHV-6 was confirmed by detection in the nails. Additional samples from the patients, such as tissue, nails and other biological samples (BALs, urine, CSF, etc.), were extracted by appropriate DNA extraction kits and tested in the same way. Viral quantity in the sample was normalised to 100,000 human genome equivalents assessed by quantification of human albumin gene.

Results: HHV-6 DNA was detected in 979 (11.5%) samples from 94 children (28.8%) and 100 adult (15.3%) patients after the HSCT in 46 children and 25 adults before HSCT. From these, Ci-HHV-6 was confirmed in 4 patients (in recipient) and 7 donors (2 more are suspected) as in engrafted post-transplant blood cells by long lasting high HHV-6 positivity and viral/human DNA ratio about 1:1. In 7 patients with Ci-HHV-6, we detected HHV-6A; in the rest HHV-6B was detected. Two patients carrying Ci-HHV-6 before alloHSCT and 4 transplanted from Ci-HHV-6 positive donor died from post-transplant complications having tissue samples tested as well.

In one patient, multiple viral infections were observed including EBV-LPD from donor Ci-HHV-6 positive cells. Patient subsequently deceased without any GvHD due to relapse of the primary leukaemia.

In total, CMV was detected in 644 patients (65.8%) from the cohort and virostatic treatment was started in 350 (35.8%) of them.

CMV resistance was detected in 20 patients (in 1, A594V was detected before the HSCT). In two Ci-HHV-6 patients, ganciclovir resistance (A594V and L595S) developed quickly after 39 and 53 days of treatment (126 and 186 days after HSCT) and so Ci-HHV-6 positive recipient seems to be at higher risk of CMV resistance development ($p < .05$).

Conclusions: As in our previous studies, we confirmed higher frequency of Ci-HHV-6A in our cohort documenting presence and quantity of HHV-6 DNA in the different biological materials and tissues. Comparing to related CMV, HHV-6 was detected less frequently. Impact of Ci-HHV-6 carriers on CMV infection seems to be in Ci-HHV-6 positive recipient only among our patients.

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Monitoring of CMV infection: A comparison of pp65-antigenemia from whole blood and Elisa in Iranian patients undergoing kidney transplantation

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Cytomegalovirus (CMV)-related disease is one of the most frequent infectious complications after kidney transplantation worldwide. Despite new therapeutic options, symptomatic CMV infection still has a high mortality rate. In countries that CMV infection is common and more than 90% of general population is CMV serology positive, PP65 antigenemia could be a good indicator of recent CMV infection. In this study, the cytomegalovirus (CMV) pp65 antigenemia assay was compared with detection of CMV IgG in 50 kidney transplant patients. Antigenemia occurred only in 5 patients (10%) a median of 30 days (range, 14–74) after transplant while all 50 kidney transplant recipients showed high level of CMV IgG. one of 5 patients who presented with positive antigenemia developed fatal CMV pneumonia 10 days later. Thus, CMV pp65 antigenemia may be useful in guiding antiviral treatment in seropositive kidney transplant recipients.

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Abstract no: 39
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Replication of porcine cytomegalovirus in mesenchymal stem cells derived from miniature pig bone marrow and lung

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Introduction: Porcine cytomegalovirus (PCMV) belongs to the genus cytomegalovirus, subfamily Betaherpesvirinae, family Herpesviridae, and is an icosahedral virus with a double-stranded linear DNA genome. The virus particle diameter is 150–200 nm. PCMV is distributed globally, with reported cases in Germany, Japan, Britain and the Unite States. Serologic survey on this virus has been reported in 76.3% of pigs but virological survey of PCMV distribution has been reported in a few papers in Korea. Cytomegalovirus have as a common feature the capacity for long-term virus persistence after primary infection, resulting in latency. PCMV grows slowly in cell cultures, and produces intranuclear inclusions in giant cells. In this study, mesenchymal stem cells (MSCs) isolated from miniature pig lung and bone marrow were infected with porcine cytomegalovirus (PCMV).

Materials and methods: Korea PCMV 47-3 strain was isolated from peripheral blood mononuclear cells (PBMCs) of a 8-weeks-old pig without obvious clinical symptoms. Miniature bone marrow derived mesenchymal stem cells (mp-BMSCs) and miniature pig lung derived mesenchymal stem cells (mp-LMSCs) were infected with Korea PCMV strain 47-3, porcine cytomegalovirus (ATCC VR-1499) and then 16 days' cultured. This culture supernatants samples were screened by PCR amplification of DNA polymerase region. Genomic DNA was extracted using DNeasy minikit (QIAGEN, USA). The primer sets (sense: 5′-CCTATGTTGGCACTGATACTTGAC-3′, anti-sense: 5′-CCCTGAAAATCACCGTCTGAGAGA-3′) were initially used to amplify PCMV gene. Finally PCMV DNA was identified by nested PCR (sense: 5′-ACGTGCAATGCGTTTTACGGCTTC-3′, anti-sense: 5′-ACTTCTCTGACACGTATTCTCTAG-3′).

Results: Cytopathic changes of mp-BMSCs and mp-LMSCs were observed 16 days post infection. Cultures with CPE were further analyzed for the presence of PCMV DNA by PCR. Different of mesenchymal stem cell was effective mp-LMSCs better than mp-BMSCs in PCMV replication.

Conclusions: In this study, MSCs isolated from miniature pig lung and bone marrow were infected with PCMV. PCMV isolation was effective mp-LMSCs better than mp-BMSCs in PCMV replication. We also provided evidence that these cells are susceptible to PCMV infection. Pigs are similar to human in anatomy, physiology and immunological responses, and thus may serve as a useful large animal preclinical model to study potential cellular therapy for human diseases. Therefore, mp-LMSCs and mp-BMSCs should be tested for PCMV before transplantation to prevent virus transmission to recipients [1,2].

Reference

[1] J.C. Booth, et al., Inclusion-body rhinitis of pigs: attempts to grow the causal agent in tissue cultures, Res. Vet. Sci. 8 (1957) 338–345.
[2] J.F.L. Fryer, et al., Quantitation of porcine cytomegalovirus in pig tissues by PCR, J. Clin. Microbiol. 39 (3) (2001) 1155–1156.

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Abstract no: 72
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Monitoring of BK and JC polyomavirus viruria and viremia in hematopoietic stem cell transplant (HSCT) and renal transplant (RT) recipients

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Background and objectives: Polyomaviruses are small, nonenveloped DNA viruses, which are widespread in nature. In immunocompetent hosts, after primary infection the viruses

remain in a latent form in the urogenital epithelial cells. Although the association of JC virus (JCV) to viral nephropathy (PVAN) is unclear, the role of BK virus (BKV) is well documented. In RT recipients, reactivation of BKV is an important cause of kidney allograft loss and the clinical manifestations range from asymptomatic replication in the urinary tract to complications such as ureteric stenosis, transient impairment of renal function, PVAN and irreversible graft failure. The classical sequence of disease is usually viruria, viremia and then nephropathy but kidney transplantation guidelines recommend BKV testing of plasma, not urine, as the most effective screening approach. In HSCT recipients, hemorrhagic cystitis (HC) is the most common complication experienced by recipients infected with BKV. Nevertheless, up to 80% of HSCT recipients are noted to have BKV viruria but only 10 to 25% of all patients develop clinically significant HC. The aim of this study was to assess the incidence of BKV and JCV infections and the diagnostic sequence influence on HSCT and RT recipients.

Methods: A long-term prospective study was conducted among patients who received a HSCT or a RT in our institution, with positive urine sample for polyomavirus and with a blood sample taken at the time of the urine sample, received in our laboratory between November 2013 and September 2015. qPCR was used to detect BKV and JCV DNA automatically extracted, with LightMix® Kit on the Roche Diagnostics LightCycler® 2.0 Instrument. PVAN was diagnosed by renal biopsy and demonstration with the immunohistochemical staining SV40T antigen.

Results: 43 patients were enrolled in the study, 27 male (62.8%), mean age 50 years old (range 11–69); 15 RT and 28 HSCT. A total of 147 samples (74 urine, 73 whole blood) were studied. The median number of samples was 1.7 urine (1–6) and 1.5 blood (1–6) per patient and the median time since transplantation to polyomavirus detection was 210.45 days (12–947). BKV or JCV viruria was detected in: 6 (40%) and 8 (53.4%) patients respectively after RT (1 patient presented both BKV and JCV, 6.7%); 17 (60.7%) and 10 (35.7%) patients after HSCT (4 patients, BKV and JCV, 14.3%). Positive viremia was found in 3 patients (7%), 2 RT (1 BKV and 1 JCV) and 1 HSCT (BKV). Among RT patients, PVAN was diagnosed in 2 patients, 1 BKV (viruria 95487 copies/ml, viremia negative) and 1 JCV (viruria $>10^6$ copies/ml, viremia 76900 copies/ml), both without any other coinfection. 10 hematologic patients presented HC, 6 ($>10^6$ copies/ml) BKV viruria, 2 ($>10^6$ copies/ml) JCV viruria, 1 (20000 copies/ml BKV and 37154 JCV viruria) and 1 (350000 copies/ml) BKV viremia and adenovirus viruria. 1 patient with HC presented BKV viremia (9268 copies/ml).

Conclusions: Although a small number of PVAN were detected, the presence of renal failure together with polyomavirus viruria should be an indication of renal biopsy in order to rule out PVAN, due to the absence of viremia in some cases. We also detected one case of JCV PVAN, which should be taken into account. Regarding the HC, viremia is not an essential factor.

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Abstract no: 77
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Torque teno virus (TTV) in immunosuppressed host: Performances studies of TTV R-Gene® kit and donors and recipients kidney samples genotyping

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Background: TTV are highly prevalent in the general population and infections are characterized by lifelong viremia. These viruses are so far not associated with clinical disease, but association of viral load variation and immunosuppression has recently been reported. Though, clarifying the role of TTV as a surrogate marker and evaluating the interest of viral load follow up to monitor patient immunity requires standardized PCR systems that accurately detect, and quantify, all the human genotypes.

We thus developed the TTV R-gene® kit (ARGENE® range, bioMérieux, France, available soon) for the detection and quantification of TTV in whole blood and studied its performances as an immunomodulation marker. TTV is also a virus with a high genetic variability, and the TTV population of genotypes, their transmission and their evolution in our patients may reflect the clinical condition of immunosuppressed patients.

Methods: Analytical studies were performed on whole blood dilutions of quantified TTV samples. The samples were extracted by NucliSENS® easyMAG® (bioMerieux) and amplified by TTV R-gene® kit with 10 μL of eluate and 15 μL of premix ready-to-use.

911 samples (from 42 kidney transplant recipients follow-up), 32 samples from donors were analyzed with the TTV R-gene®. 66 samples of kidneys donors and recipients (paired) were preprocessed and extracted before RCA amplification and purification. The libraries were prepared with Ion express library kit and sequenced by Ion Proton high throughput sequencing technology (ion Torrent, Life technologies).

Results:

LoD: The limit of detection in whole blood determined by Probit analysis is 148 cp/mL of sample on whole blood.

Linearity: Linearity of the quantification results is demonstrated over the range $2.1 \times 10E+3$ to $4.1 \times 10E+10$ cp/mL.

Specificity: All TTV genotypes were correctly detected and quantified in whole blood samples with TTV R-gene®. The following viruses were not detected with TTV R-gene® kit: animal TTV, CMV, EBV, BK, Adenovirus, HSV1/2, VZV, HHV6/7/8 and B19.

Precision: The coefficient of total variability ranged between 2.6% (upper limit) and 11.4% (lower limit).

TTV viral load follow up: TTV was detected from 75% of donors samples. Viral load ranged from 1.54 log to 5.59 in donors. In graft recipients, the overall prevalence of TTV in blood was 95.2%. All TTV positive patients raised their TTV load from an average of 5.34 log to 8.89 in the first 75 days post transplantation.

Donors and recipient sample sequencing: the preliminary analysis of the first sequencing chip (18 samples) showed the presence of many TTV genotypes. On average, there are 2.1 million of reads/sample.

Final sequencing results of all samples (genotypes and prevalence) will be presented with the goal to study the link between detected TTV genotypes and others viruses. Biological and clinical data will be correlated.

Conclusion: The high quality of the TTV R-gene® kit in terms of analytical sensitivity, specificity, linearity and precision was demonstrated. Due to a harmonized protocol and format, this kit can be used in the same workflow that other R-gene® kits for transplanted patient monitoring potentially adding a tool to monitor the patients immune status. The analysis of TTV genotypes present in kidneys of donors and recipients by sequencing adds useful information to increase our knowledge about the correlation of TTV viral loads and immune status of immunosuppressed patients in order to improve individual treatment.

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Abstract no: 79
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Rapid adenovirus typing method for species identification

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Adenoviruses are characterized by a large variability, reflected by their classification in species A to G. Certain species, e.g. A and C, could be associated with increased clinical severity, especially in immunocompromised hosts. Hexon sequencing represents the most common method for Adenovirus typing. However, Adenovirus capsid protein VI is characterized by significant interspecies nucleotidic and gene size variability, which is conserved within a given species and thus enables species differentiation.

Our group therefore designed a "pVI rapid typing method" to obtain rapid and easy species assignment for Adenoviruses, thanks to combined fusion temperature (Tm) and amplicon size analysis. The method was established using plasmids encoding cloned protein VI genes from different Adenovirus species. In a second step, the Typadeno study was started to compare the "pVI rapid method" results to hexon Sanger sequencing results in 140 Adenovirus-positive clinical samples.

In a subsample of 55 samples with results for both tests, species A and C could be identified with a 100% positive predictive value (95% confidence interval of respectively [39.76–100.00%] and [83.89–100.00%]), thus confirming the potential value of this simple typing method.

Our study is the first giving promising results for this new technique which would need to be confirmed in a larger assessment.

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Abstract no: 12
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Genotyping and full genome sequencing of varicella–zoster viruses isolated from Korean patients

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Varicella–zoster virus (VZV) is a causative agent for chickenpox in primary infection and shingles after reactivation from latency. Live attenuated vaccines have been developed based on Japanese Oka strain and Korean MAV/06 strain. A number of complete or near complete genomic DNA sequences have been determined for genetic analyses. Recently, 3 clinical strains were isolated from Korean patients and their genome sequences were completed by high through-put sequencing technology. In this study it was attempted to analyze the single nucleotide polymorphism (SNP) of the VZV strains in order to understand the characteristics of Korean clinical isolates. Phylogenetic analyses with 42 non-vaccine and independent VZV strains including the 3 Korean strains YC01, YC02 and YC03 placed the 3 Korean strains to the clade 2 together with pOka and LAX1. Comprehensive SNP analyses identified 87 sites specific for each of the 5 VZV clades. Clade 2 could be further divided into 2 subclades: subclade 2a including pOka, LAX1 and YC01, and subclade 2b including YC02 and YC03. Subclade 2a and 2b differed at 6 SNP sites. The subclade 2b strains YC02 and YC03 also shared similar bootscanning pattern distinct from the bootscanning pattern of the subclade 2a strains. The subclade 2 strains appeared to descend earlier than the subclade 2 strains from the most recent common ancestor of the clade 2.

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The distribution of enteroviruses isolated in virology laboratory, Singapore General Hospital, 2008–2015

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Introduction: A study on the distribution of enteroviruses was conducted to get a better understanding of the epidemiology of enteroviruses in Singapore. The data were from 2008–2015, when Singapore experienced several hand, foot and mouth disease (HFMD) epidemics caused by various viruses predominating in different years.

Method: Laboratory data for patient samples sent for enterovirus culture from 2008–2015, stored on the laboratory information system (LIS), were retrieved and analysed. Laboratory data comprised the test result, age, and gender of the patients.

Results: Out of 1890 samples comprising stool, rectal swabs, throat swabs, oral ulcer swabs, autopsied heart and intestinal tissues, 173 enterovirus isolates were obtained. There were 21 (28.8%) isolates from patients aged <2 months, 101 (58.4%) isolates from patients aged 2 months to <3 years, 31 (17.9%) isolates from patients aged 3 to <7 years, 15 (8.7%) isolates from patients aged 7 to <15 years, and 5 (2.9%) isolates from patients 15 years and older. A male predominance (65.9% male, 34.1% female) was observed. There were 22 enterovirus serotypes identified. Majority (74%) of the isolates were members of the species human enterovirus A, which include the serotypes EV-71 (55, 33%), CV-A6 (31, 19%), CV-A10 (17, 10%), and CV-A16 (13, 8%), which are associated with HFMD. The remaining (26%) were members of the species human enterovirus B, which include E19 (9, 5%) and CV-B5 (8, 5%).

Out of 1269 cerebrospinal fluid (CSF) and autopsied brain tissue examined, 16 enterovirus isolates were obtained from patients aged 11 days to 40 years. There were 12 (75%) isolates from patients aged <3 months. No gender predominance (50% male, 50% female) was observed. There were 10 enterovirus serotypes identified. The predominant serotype was CV-B5 with 5 isolates (31.3%).

Conclusion: The predominant serotypes isolated are those commonly associated with HFMD, due to the laboratory's participation in Singapore's Ministry of Health HFMD surveillance programme. Thus, unlike reports from Marseille and the United States where HEV-B species account for the majority of isolates, this study shows a predomination of HEV-A species. The largest number of isolates was obtained from the age group eligible for enrolment in infant/child care centres prior to entering pre-school (2 months to <3 years). This reinforces the importance of infection control in care centres with infants and young children to minimise the spread of infectious diseases. Currently, Singapore's enterovirus surveillance only includes HFMD, poliovirus and enteroviral encephalitis. It might be beneficial to conduct routine surveillance of enteroviruses, similar to the National Enterovirus Surveillance System (NESS) in the United States, to document disease associations, and provide samples of new viruses for study.

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Abstract no: 147
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Prevalence of enterovirus in patients with meningitis: 2007–2015

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Objectives: Viruses are the major cause of the acute meningitis. Nonpolio enteroviruses (%80–95) account for most of the aseptic meningitis cases for which an etiologic agent is identified. The aim of this study was to establish the prevalence of enteroviruses in patients with aseptic meningitis in our region.

Methods: Between January 2007 and December 2015, cerebrospinal fluid (CSF) specimens were collected from 576 [250 (43.4%) female, 376 (56.6%) male] paediatric and adult patients who admitted with meningitis symptoms in Ege University Faculty of Medicine Hospital. The age range of patients is between 21 days to 90 years (median: 11 years). Of the 576 patients, 375 (65.1%) were paediatric, and 201 (34.9%) were adult patients. Shell vial cell culture method was performed for all the specimens sent to cell culture laboratory. After specimens were vortexed, three shell vials were prepared for each patient [Human laryngeal carcinoma (HEp-2), embryonic rhabdomyosarcoma (RD), and African green monkey kidney (Vero) cells line]. Each vial was inoculated with 0.5 ml CSF for the recovery of enteroviruses. The vials were centrifuged at 700 × *g* for 30 min at 25 °C and incubated at 37 °C for 1 h. Supernatants were aspirated from each vial. Subsequently, 1 ml isolation medium containing Eagles MEM supplemented with 2% FCS and antibiotics was added to the vials containing HEp-2, RD, and Vero cells. Then, the vials were incubated in moist chamber at 37 °C in a 5% CO_2 atmosphere for 48 h. Coverslips were fixed and stained with a fluorescein isothiocyanate (FITC) labelled polyclonal antibody specific for enteroviruses (Pan-Enterovirus Blend, Chemicon International, USA) according to the manufacturer's protocol. The coverslips which had one or more fluorescing inclusions bodies were considered as positive.

Results: In 576 patients, 35 (6.1%) patients were positive for enteroviruses. Of the 35 patients (17 female, 18 male), 10 were adult (median: 35 years) and 25 were paediatric (median: 8 years) patients. All the patients that are enteroviruses positive had the clinical diagnosis of aseptic meningitis except one patient who has encephalopathy.

Conclusion: Most of patients (71.4%) which enterovirus has been detected were paediatric patients. Aseptic meningitis was the most common clinical diagnosis. The overall prevalence of enteroviruses was 6.1% in our region.

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Abstract no: 163
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Controlling the quality of diagnostic PCR assays

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The aim of infectious disease diagnostics is the early detection of infection to allow for the administration of appropriate therapy. Quantitative real time PCR (qPCR) has revolutionised the fields of pathogen detection by offering faster, more accurate and more sensitive results than traditional methods. However qPCR is not without problems, principally of assay performance.

It is essential that laboratories monitor the day-to-day performance of an assay, specifically with respect to reproducibility and repeatability, so that the laboratory may be confident that the results generated by the assay lead to appropriate diagnoses. Frequently laboratories do not run external standards which would allow for easy performance monitoring. This is often due to the unavailability of appropriate controls and an unavailability of a means of monitoring assay results. NIBSC have sought to address these issues in two ways.

First, NIBSC have produced a series of CE-marked multiplex run control reagents for qPCR. These reagents are formulated with intact viruses and therefore can be used as a whole process controls. The concentrations of all of the analytes in the reagents are set at a level designed to be challenging to an assay but to be consistently detected as low positives. The controls are available freeze

dried and calibrated where possible to the appropriate International Standard.

Second, NIBSC have developed a web based Results Reporting System (RRS) to data monitor both serology and PCR reagents. The system allows for real-time comparison of both intra- and inter-laboratory results by provision of Levey–Jennings plots and indicates when an assay is falling out of specification. By application of Westgard Rules to the data, any deviations from the norm can be quickly recognised and addressed.

Medical Diagnostic Laboratories who are audited against ISO 15189 are required to use "suitable reference material" and where samples are tested at different laboratory sites there should be a mechanism to "verify the comparability of results". Use of the reference materials provided by NIBSC together with the RRS allow a medical diagnostic laboratory to meet the requirements of ISO 15189.

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Abstract no: 206
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Evolutionary studies of herpes simplex viruses (HSV) genomes provide evidences of HSV-2/HSV-1 interspecies recombination

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Herpes simplex virus 2 (HSV-2) is a prevalent sexually transmitted infection responsible for recurrent genital lesions and may also cause neonatal morbidity and mortality. The average prevalence is around 11% but important regional variations exist, with the highest prevalence observed in sub-Saharan Africa (31.5%) [1]. HSV-2 generally exhibits low genomic variability. The maximum overall divergence is only 0.4% and most open reading frames (ORF) exhibit little, if any, variability [2]. Recently, we described an HSV-2 variant mainly found in sub-Saharan African individuals characterized by highly divergence among UL30 gene (maximum divergence of 2.4%) [3]. In order to clarify the evolutionary history of this variant, sequences of nearly complete genomes were obtained from 18 isolates of HSV-2 variant recovered from distinct patients originating from Africa.

Sequencing libraries were prepared using extracted DNA from supernatants of infected cell cultures, then were subjected to in-solution hybridization capture and sequenced on a MiSeq® platform (Illumina) with a resulting average coverage of the genome sequences about 75%. Whole genome sequence comparisons revealed unexpected diversity, with many sequences exhibiting more than 0.7% pairwise divergence. Phylogenetic analyses identified two main lineages: a previously unrecognized African lineage, mostly comprising sequences originating from sub-Saharan Africa, and a worldwide-spread lineage, even distributed in sub-Saharan Africa. Recombination analyses performed thereafter notably evidenced that members of both lineages could recombine among themselves. Moreover, those analyses also showed that interspecific recombination might have occurred between HSV-2 and HSV-1 ORF fragments, as evidenced for UL29 and UL30, and, to a lesser extent, for UL15 and UL39. The recombination status at these loci was used to investigate the relative timing of the recombination events. While the recombination events in UL15 and UL39 appeared after the African and worldwide-spread lineages started their diversification, the recombination events in UL29 and UL30 likely constituted the milestone of the worldwide-spread lineage origin.

Those results highlight the potential African origin of HSV-2, which is coherent with human species evolutionary history, and assess, unlike to common belief, the occurrence of interspecies HSV-2/HSV-1 recombination under natural conditions [4]. The extended gene flow from HSV-1 into HSV-2 genomes may have contributed to the rise of the worldwide-spread lineage. Further investigations are now required in order to determine whether HSV interspecific recombination is still an ongoing process and has any clinical implications.

Reference

[1] K.J. Looker, A.S. Magaret, K.M. Turner, P. Vickerman, S.L. Gottlieb, L.M. Newman, Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012, PLOS ONE 10 (2015) e114989.
[2] R.M. Newman, S.L. Lamers, B. Weiner, et al., Genome sequencing and analysis of geographically diverse clinical isolates of herpes simplex virus 2, J. Virol. 89 (2015) 8219–8232.
[3] S. Burrel, N. Desire, J. Marlet, et al., Genetic diversity within alphaherpesviruses: characterization of a novel variant of herpes simplex virus 2, J. Virol. 89 (2015) 12273–12283.
[4] E. Thiry, F. Meurens, B. Muylkens, et al., Recombination in alphaherpesviruses, Rev. Med. Virol. 15 (2) (2005) 89–103.

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Commutability and agreement of international and secondary standards

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Background: Quantitative testing of viral loads has become an integral part of care for immunocompromised patients. Information on the commutability of quantitative assays is currently not available due to differences in methodology, chemistry and equipment. The WHO international standards and secondary standards have been developed for only a few viruses associated with these immunocompromised patients, aiming to improve information on commutability and to standardise diagnostics between laboratories. This study aims to investigate the commutability of international standards, as well as to evaluate several secondary standards.

Material/methods: WHO international standards as well as commercially available secondary standards were compared using real-time PCR as well as digital PCR technology. We investigated the commutability of international standards and the agreement between several secondary standards for the same targets. Cytomegalovirus, Epstein–Barr virus, BK virus, Varicella Zoster Virus, Hepatitis A virus, Herpes Simplex virus type 1 and 2 were included.

Results: The international as well as secondary standards indicated a high correlation and suitability to improve diagnostics. WHO international standards for Cytomegalovirus and Epstein–Barr virus showed significantly. However, when no international standard is present for a target, the agreement between secondary standards was significantly lower. The data collected

were subsequently used to understand their value in those instances where no standard was available.

Conclusions: The WHO international standards contribute to the standardisation of quantitative diagnostics. However, this study shows that they are currently not commutable. In some cases, a secondary standard is available for viral targets which still lack a primary and WHO accepted international standard; agreement between suppliers is low however. In the absence of international standards for a large number of (viral) targets, new strategies can and must be used to improve this lack of standardisation. We theorise that when optimal diagnostic assays are used, a target-independent universal standard can be implemented.

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Abstract no: 326
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Half of family doctors do not use the flu vaccine

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Questionnaires about Flu Vaccine on Primary Health Care Personnel Knowledge, Attitude and Behavior were administered to 180 Health Care Workers. 27.2% of respondents fever and headache, arthralgia and 22.2% stated that the runny nose is the most obvious symptom of the flu. Flu a few times the last 3 years the rate of 41.11% while the pass rate of only 23.88% who had once been identified as influenza. Those who say I never pass rate is 13.33%. The rate of influenza using the rest spend the last 3 years has been seen as 77.22%. Participants in most patients with cough indoors flu, with close contact with the patient and handshaking and kissing, stated that transmitted by close social contact. I place plenty of fruits and vegetables to prevent the flu, herbal tea drink, as I have stated Avoid contact with patients. "Did you make a flu vaccine in the past five years?" Is the question that is 49.44% of family physicians ever make, the 27.22's% and 23.33% for each year that they stated that they only once. 63.33% of respondents think that the only necessity of the vaccine in special cases, the 12.22% stated he thought it was unnecessary. 30% of respondents stated that the flu vaccine last 3 years even though the flu was built. The ratio of non-believers that the vaccine is protective is determined as 35%. A participant recommended flu vaccine for health workers, often those with the flu, the elderly, children, and with chronic illness. "Flu vaccine is given simultaneously with other vaccines" is no question as to 71.11%, 13.88% gave the answer is yes.

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Prevalence of specific IgGs against the study Rubella Virus in Sao Tome and Principe with "Guthrie Cards"

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Introduction: Rubella it's a contagious mild viral disease that usually affects children. In pregnant woman, the fetal spread can origin miscarriage or congenital rubella syndrome. São Tomé e Príncipe is one of the countries where rubella vaccination is not established. Some seroprevalence studies to other viruses have used "Guthrie cards" (DBS), since they are easily collected, readily stored and require minimum quantity of capillary blood.

Objective: Determination of rubella IgG seroprevalence in a São Tomé e Príncipe population using DBS, validating previously this method.

Material and methods: Population: DBS from 173 females and 143 males from São Tomé e Príncipe (ages between 2 and 35 years) were collected between January and May of 2014.

- IgG extraction method: Elution of 32 mm^2 DBS (1 h/600 rpm, followed by 16 h/4 °C).

IgG determination: SERION ELISA classic Rubella Virus IgG. Method validation: Sensitivity and specificity were evaluated using the same ELISA technique in serum as reference.

Results: Method validation: Sensitivity: 89%; specificity: 100%.

Of the 316 analyzed samples we obtain 95 negative (<10 UI), 203 positive (>15 UI) and 18 borderline (10–15 UI). The IgG's prevalence against rubella virus in São Tomé e Príncipe was 65%. The prevalence was similar throughout the island.

Conclusions: In our study DBS showed compatible results with the reference method, suggesting that they can be used for epidemiologic surveillance in regions with limited laboratory resources. The high rubella seroprevalence noticed in São Tomé e Príncipe also suggests that natural infections are very common in this area and can be responsible for serious clinical consequences (congenital rubella syndrome) in this population.

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Abstract no: 4
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Investigation of HSV1 positivity of patients with neurological symptoms by real time PCR method

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Introduction: Herpes simplex virus type 1 (HSV-1) is a highly infectious virus which is neurotropic and highly prevalent. The majority of HSV-1 infections occur during childhood and infection is never cleared, with lifelong potential for symptomatic or asymptomatic viral shedding episodes. In rare cases, infection can lead to more serious complications, such as encephalitis. Herpes simplex encephalitis is the most common of the sporadically occurring forms of viral encephalitis. The aim of this study was detection of HSV-1 DNA in patients applying to our hospital with neurological symptoms by real time PCR, retrospectively and investigation of the association between HSV-1 and clinical characteristics.

Material and methods: Totally 152 patients (77 female, 75 male), between 0–82 years old, who applied to Department of Neurology, Infectious Diseases and other clinics in Gazi University Faculty of Medicine, Molecular Microbiology Laboratory between April 2014–January 2016 were included in this study. DNAs were extracted from blood and CSF samples that were sent to our laboratory by spin-column method (High Pure Viral Nucleic Acid Kit, Roche, Germany). Amplification was done by Real Time PCR method (LightCycler® HSV1/2 Qual Kit, Roche, Germany) in Light Cycler 2.0 (Roche, Germany) device and the results were evaluated qualitatively. HSV-1 IgM and HSV-1 IgG antibody titres of the patients were studied by ELISA (DIA. PRO, Milan, Italy).

Results: Totally 166 samples were composed of 83% (138/166) CSF and 17% (28/166) blood. Sixty five percent (108/166) of the samples were send from neurology and the others 13%, 10%, 8% and 4%, paediatrics, infectious diseases clinics, intensive care units and other clinics respectively. HSV-1 DNA was found positive for 6% (10/166) of the samples while all samples were negative for HSV-2 DNA. Two of 7 positive CSF samples were belong to same patient and the patient's blood sample was also send to our laboratory at the same time, hence 5% (8/152) of patients were HSV-1 DNA positive. HSV-1 IgM was positive for 1% (2/152) and HSV-1 IgG was positive for 8% (12/152) of the patients. Three (37.5%) of positive patients hospitalized at neurology clinics, 3 (37.5%) of them were from various intensive care units, 1 (12.5%) was from paediatrics and 1 (12.5%) was from paediatric infectious diseases clinics. Three (37.5%) of positive patients were associated with Herpes simplex encephalitis, the other positive patients had meningitis, Wilson disease, Guillain Barre syndrome, multiple sclerosis and immunodeficiency (autoimmune haemolytic anaemia) diagnosis.

Conclusion: Real time PCR is a reliable and sensitive method for early diagnosis of viral infections which are quite important in central nervous system infections. The results are obtained in a short time through this method and because of it is very sensitive, low positive results can be detected. Thus early treatment prevents sequels. There are studies about the association of HSV-1 and Multiple sclerosis, Guillain Barre Syndrome and other neurological disorders. Therefore, we consider that using real time PCR method can be sensible for the patients with variable neurological disorders like the patients with encephalitis suspicion.

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Abstract no: 105
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Chickenpox exposure in pregnancy – A comparison of a qualitative and a quantitative varicella zoster virus antibody assay and follow up of patient outcome

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Background: Public Health England recently issued new guidance on how to best use tests for varicella zoster virus (VZV) antibody when deciding who should receive VZ immunoglobulin (VZIG) after exposure to VZV. Among pregnant women, testing is restricted to those who do not have a history of chickenpox as commercial antibody assays lack sensitivity. This includes one of the most widely used, the qualitative VIDAS VZV IgG assay (BioMérieux). When qualitative assays produce negative or equivocal results, the decision to recommend VZIG should now be based upon the result of a quantitative antibody test. The level of VZV IgG deemed protective has been set at 100 mIU/ml. As a result of this guidance we decided to compare the clinical utility of a negative VIDAS antibody result with the result of a new quantitative VZV IgG assay (Alegria – Orgentec) when advising on VZIG administration.

Methods: The audit was of the outcomes of pregnant women, exposed to chickenpox, who tested VZV IgG negative by VIDAS between 2013 and 2015. Stored serum samples from these patients were re-tested using the Alegria. This assay categorizes samples as follows: VZV IgG <50 mIU/ml (negative), 50–100 mIU/ml (borderline) and >100 mIU/ml (positive). Information regarding administration of VZIG, development of a rash and follow-up VZV IgG results was collated for the patients.

Results: A total of 29 pregnant women were re-tested by Alegria; 10 women had an antibody level of >100 mIU/ml, 10 had borderline results (50–100 mIU/ml) and 9 had negative results (<50 mIU/ml). Infection with VZV, defined as either development of chickenpox or seroconversion, was seen in only 4/29 (14%) and was confined to those with an antibody level <50 mIU/ml. On follow-up of these 4 patients, 1 developed a typical chickenpox rash, 1 seroconverted by both VIDAS and Alegria assays, but 2 developed only a borderline antibody response by Alegria and were still negative by VIDAS at follow-up. All four of the women with evidence of VZV infection had been exposed to their own child with chickenpox. This contrasts with the other 5 women with an antibody level <50 mIU/ml, with no evidence of virus transmission who were exposed only during the 1–2 days before rash onset. The mean time to administration of VZIG was the same in both groups (5.6 days vs 5.8 days). Of the 20 women who were either antibody positive ($n = 10$) or borderline ($n = 10$) by Alegria, none developed chickenpox. This included six with antibody positive and four with borderline results who had elected not to receive VZIG because of previous childhood exposures which had not resulted in chickenpox.

Conclusions: This study highlights the importance of test choice when assessing VZV immune status, with 10/29 (34%) of our patients negative by a qualitative assay, having antibody levels >100 mIU/ml when tested by a quantitative assay. Secondly, given that none of our patients with antibody levels of 50–100 mIU/ml developed infection, even when VZIG was not administered, we

propose that a VZV IgG level of 100 mIU/ml as a cut-off for immunity is too high. Finally, of our antibody negative patients, all of those exposed to the rash were infected, whilst none of those exposed before the rash were. This provides evidence against the currently held dogma that chickenpox is infectious pre-rash and certainly demonstrates the need for further study on this topic.

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Abstract no: 219
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Low risk of wild poliovirus importation to Germany via asylum seekers from polio-risk countries: Results of stool screening and serology, 2013–2015

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Poliomyelitis is a highly infectious, vaccine-preventable disease caused by poliovirus (PV) 1, 2, or 3 for which there is no cure. While only one in about 200 infections results in typical acute flaccid paralysis, most infected people (>95%) have no or unspecific symptoms. These persons may still shed virus, thus posing a risk to unprotected contacts. Since the Global Polio Eradication Initiative was launched in 1988, polio cases have decreased by over 99% to 74 reported cases in 2015 – an historical low. To date, polio remains endemic in only two countries: Afghanistan and Pakistan. However, polio can easily spread from these to other countries which are still vulnerable to PV outbreaks due to vaccination gaps, weak health systems, poor sanitation, insecurity, and refugee flows. Therefore, the World Health Organization declared the international spread of wild poliovirus a Public Health Emergency of International Concern (PHEIC) in May 2014. In Germany, the number of asylum seekers has increased since 2013, with the largest group coming from Syria experiencing a wild PV outbreak at this time. In order to estimate the risk of wild PV importation, a stool screening for PV was initiated in asylum seekers from Syria. Furthermore, a seroprevalence study was performed testing sera of persons from polio-risk countries.

(1) From November 2013 to April 2014, 118 refugee centres and public health offices from all 16 German federal states were sending stool samples for PV diagnostics to the National Reference Centre for Poliomyelitis and Enteroviruses at RKI (NRZ PE) and three labs of the German enterovirus laboratory network (LaNED). Samples from 629 asymptomatic Syrians (71% aged <3 years) were tested using molecular and virological methods. Of these, 92 (14.6%) were enterovirus-positive. Vaccine-like PV strains were detected in 12 persons indicating a recent polio immunization (OPV). Wild PV were not identified.

(2) From May to July 2015, 587 sera from asylum seekers from Afghanistan, Iraq, Pakistan, Somalia, and Syria were examined for PV neutralizing antibodies at the NRZ PE. The median of age in the study group was 25 years (12–68 years). For all three PV types, the following seroprevalence rates were found: PV1: 96.8%, PV2: 99.5%, PV3: 91.5%. Independent from age and country of origin, protecting antibodies were detected in at least 93% for PV1 and PV2 and 89% for PV3 of asylum seekers examined.

Results of the stool screening indicated a low risk for wild PV importation by asylum seekers. Due to OPV vaccination campaigns implemented in Syria and neighbouring countries, the presence of vaccine-like PV in asylum seekers was expected. Results of PV antibody testing in asylum seekers from five polio-risk countries demonstrated a high seroprevalence against all three PV types, similar to the population living in Germany. Therefore, a general screening for PV shedding or PV antibodies is not recommended. In case of unknown vaccination status, asylum seekers should be vaccinated according to the existing recommendations of the German Standing Committee on Vaccination, giving priority to children.

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Abstract no: 274
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National serological survey – Portugal 2015–2016: Study design

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Background: The Portuguese National Vaccination Program was implemented in 1965. During the last decade, the vaccine coverage in Portugal was higher. However, there are local asymmetries that may result in pockets of susceptible persons.

Other communicable diseases related to sexually transmitted agents are considered a major problem in Public Health (PH), also in Portugal, namely HCV, HIV, Syphilis and Chlamydia infections.

In Portugal, national serological surveys have been conducted on irregular basis and the lack of essential epidemiological information to support PH decisions has been considered a major concern. A national serological survey is an effective way to fulfil these needs.

Aim: To describe the study design of the National Serological Survey 2015–2016, an ongoing study to assess the prevalence of antibodies regarding Vaccine Preventable Diseases (VPD) and other infectious diseases, with major impact in Public Health.

Material and methods:

Type of study: A national cross-sectional study has been planned with one time specific specimen collection per case. Participation in the study is voluntary and comprises a signed informed consent statement, answering a questionnaire and collection of biological specimens.

Infectious agents studied: Samples collected will be tested to the following agents: *Bordetella pertussis, Haemophilus influenzae* type b, *Corynebacterium diphtheriae, Clostridium tetani*, HBV, Measles virus, Mumps virus, Rubella virus and Poliovirus. Additionally are tested for HCV, HIV, *Treponema pallidum and Chlamydia trachomatis*.

Estimated sample size: Prevalence data from the previous NSS was used for VPD sample size estimation. Sample has been stratified by 8 age groups (starting age = 2 y/o), equally distributed by gender and assuming at least a precision of 5% and a design effect of 1.5. To ensure regional representativeness of the population, estimated sample size was set up to 4543 individuals.

Sample size for HCV, HIV and Syphilis antibodies prevalence was set up to 2884 individuals (stratified in 5 age groups; starting age = 18y/o) and for *Chlamydia trachomatis* detection in a total of 1152 individuals (stratified in 2 age groups; starting age = 18y/o). Sample size for these agents was calculated assuming 50% prevalence with 5% precision and considering a design effect of 1.5.

Laboratory tests and data analysis: Poliovirus antibody detection will be performed by neutralizing test. Specific antibody detection for the other agents is performed by immunoassay methods. *Chlamydia trachomatis* detection in urine will be carried out by real time PCR.

Data from questionnaires and test results is entered into a RedCap database by double-input. The statistical analysis will be descriptive.

Expected results: Determination of the prevalence for all infectious agents enrolled in the current study, analysed by gender, age, geographic distribution, and also regarding the socio-economic factors.

Expected outcomes: The expected outcomes are to improve the knowledge on the immunity profile of the Portuguese population concerning VPD and provide population-based health information to support the evaluation of the current National Vaccination Program and also the implementation of national guidelines regarding the prevention of sexually transmitted infections.

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Abstract no: 275
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Mobile phones of paediatric senior physicians are highly contaminated with viral genomes of several epidemic viruses during the winter season

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Background: Mobiles phones (MP) routinely used by health care professionals are potential reservoirs of nosocomial bacteria, but few data are available concerning viruses. A first study performed in our centre (Pillet et al., Clin Microbiol Infect 2016) that recorded the contamination by viral genome at a single day showed that rotavirus RNA can be detected on MP, notably in paediatric emergency and paediatric departments.

Objectives: To correlate the circulation of epidemic viruses with the contamination by RNA and DNA viruses on MP used by paediatric senior physicians during all the winter season.

Study design: Ten digital enhanced cordless telephones (DECTs) were selected and wiped each week from December 15 2015 to May 3 2016 with a 480CE e-swab (Copan, Brescia, Italy). The swabs, placed in transport medium, were frozen at −80 °C before virological analysis. A volume of 200 μL of transport medium was extracted by using the Specific B protocol on the NUCLISENS easyMAG instrument (bioMérieux, Marcy l'Etoile, France) under an elution volume of 50 μL. Respiratory viruses (respiratory syncytial virus (RSV) A et B, influenza A et B, adenovirus, metapneumovirus, coronavirus 229E, NL63 and OC43, parainfluenza virus 1, 2, 3 and 4, bocavirus, enterovirus and rhinovirus) and gastro-intestinal viruses [norovirus GI and GII, rotavirus A, adenovirus F (serotype 40/41), astrovirus and sapovirus] were detected by RT-qPCR by using Anyplex™II RV16 Detection kit and Allplex™ Gastrointestinal Full Panel Assay (Seegene, Eurobio, Courtaboeuf, France) respectively, according to the manufacturer's instruction. A questionnaire was filled-in by the professionals before and after the study in order to record their behavioural pattern in the use of MP and the hygienic measures that they apply to these devices.

Results: Among the 220 collected swabs, 110 (50%) were positive for at least one viral genome. A total of 181 viruses were identified. Multiples contaminations (up to 4 viruses) were described on 53 swabs (48.2%). Adenovirus, bocavirus and rotavirus, genomes were the most frequently detected on professional MPs, with 71, 60 and 28 specimens found positive, respectively. Adenovirus F, RSV-A, coronavirus OC43, coronavirus NL63, rhinovirus and enterovirus genomes were less frequently detected. The contamination of the MPs was correlated to the viruses circulating the week before sampling. Half of the professionals declared to clean their phone at least once-a-week at the beginning of the study and all increased the frequency of cleaning at the end of the study.

Conclusions: These results suggest that professional MPs used by paediatricians are the vectors of a large panel of epidemic viruses that circulated during the period study and may participate to the dissemination of viral outbreaks in paediatric settings. As MPs are not considered as medical devices, no formal recommendations are currently available concerning hygienic measures related to their disinfection. Promotion of MP cleaning together with hand hygiene surrounding the use of MPs is urgently needed.

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National serological survey – Portugal 2015–2016: Rubella seroprevalence in a population-based sample of childbearing age women resident in the North, Lisbon and Algarve

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Background: In Portugal, three different strategies for prevention of congenital rubella have been implemented. The first started in 1982 with the immunization of adolescent girls (aged 11–13 years) and non-immune women. The second began in 1987 with a single dose of measles, mumps and rubella vaccine (MMR) for boys and girls aged 15 months, followed by the introduction in 1990 of the two MMR doses at 15 months and 11–13 years respectively.

Presently, rubella vaccine is routinely available as MMR; the first dose is given at 12 months and the second at 4–6 years of age.

Aim: Determination of rubella IgG seroprevalence in a representative sample of women aged 20 to 44 years residents in three territorial units (NUTII) of Portugal mainland.

Material and methods: *Study population*: The population-based sample of women at childbearing age was collected in the framework of the project National Serological Survey – Portugal 2015–2016 (project funded by Iceland, Liechtenstein and Norway through the EEA Grants) and was calculated to be nationally representative and corresponding to 42 women in the North and Lisbon

NUTII regions and 43 in Algarve. In each NUTII the sample was divided in 2 age groups: 20-29 years old and 30 to 44 years old.

Methods: Rubella specific IgG was detected in sera by enzyme-linked fluorescent immunoassay (ELFA) using commercial kits (VIDAS® RUB IgG ii). The positive limit for this method is 15 IU/mL Sera with results between 10 and 14 IU/mL should be interpreted as equivocal and as negative those with values below 10 IU/mL.

Statistical analysis consisted in the determination of absolute and relative frequencies.

Results: Of the 127 women studied 98 (77.2%) are Portuguese origin 18 (14.2%) were not born in this country, and 11(8.6%) did not answer this question. From these 122 (96.1%) had IgG antibodies (≥15 IU/mL) to rubella virus, 1 (0.8%) present an equivocal result (10 IU/mL to 14 IU/mL) and 4 (3.1%) were negative (<10 IU/mL).

The distribution of the number of women with a positive result by NUTII showed a percentage of 95.2% ($n = 40$) seropositive women to North and Lisbon and 97.7% ($n = 42$) to Algarve.

The results were also analyzed by age group taking into account the history of rubella vaccination in Portugal. The age group 20–29 years old correspond to women that must have been vaccinated with two doses of MMR vaccine while women aged 30 to 44 years old includes those who may have been immunised with MMR, those who were vaccinated between 11 and 13 years old and those who were naturally infected. This analysis showed that women aged 20 to 29 years old have a seroprevalence of 100% (33/33) to rubella virus while the women aged 30 to 44 years old presented 94.7% (89/94).

Discussion and conclusion: The results of this study showed a high seroprevalence to rubella virus in women of childbearing age and are similar to the results of the previous NSS 2000–2002. The high seroprevalence found can be associated to values of vaccination coverage and effectiveness of rubella vaccine.

The NSS 2015–2016 is still ongoing and samples from others regions and age groups are being collected and studied. A more detailed analysis will be made after completion of the study. However despite these results, it is advisable that the knowledge of rubella immune status before conception and possible vaccination prior to pregnancy continues to be a recommended procedure in order to prevent and eliminate congenital rubella.

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Abstract no: 128
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Automation of Luminex NxTAG respiratory pathogen panel

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Background: Diagnostic laboratories more and more shift from traditional testing methods to faster, more sensitive, and more cost-effective molecular methods because there is a continuous desire to maximize productivity, improve workflow, optimize staff time, and reduce the time to deliver results back to healthcare providers. The novel Luminex NxTAG Respiratory Pathogen Panel (RPP), which was recently CE-IVD marked, is a qualitative nucleic acid multiplex test that provides simultaneous detection and identification of 19 viruses and 3 atypical bacteria associated with respiratory tract infections. It is a ready to use system requiring very little hands-on time and is performed in a closed PCR vessel, reducing the chances of contamination. Nucleic acid is simply added directly to pre-plated lyophilized reagents for RT-PCR and bead hybridization.

In this study the extraction part was further automated on the Hamilton Microlab STAR platform and compared to the semi-automated easyMAG extraction as outlined in the package insert. Assay performance, hands-on time and overall turnaround-time was compared.

Material/methods: Contrived samples were created by suspending American Type Culture Collection (ATCC) and Zeptometrix control strains in Remel Micro Test M5 media, which were then added to BD UVT to prepare a moderately positive sample for extraction. A total of 6 replicates of each sample were extracted across five Microlab STAR runs and subsequently tested using the NxTAG Respiratory Pathogen Panel. The same samples extracted on the easyMAG instrument and tested using NxTAG RPP were used as the comparator method.

Results: Microlab STAR and easyMAG workflows were evaluated for 24, 48, 72 and 96 sample batches. The Microlab STAR reduced hands-on time by 30.22, 68.08, 103.95, and 141.81 min respectively. While total assay time was slightly higher for the Microlab STAR when running 24 samples compared to the easyMAG, actual hands-on time by the user was reduced by 31.22 min. Total assay time and hands-on time were reduced when comparing 48, 72 and 96 sample run sizes on the Microlab STAR to a single easyMAG. Samples extracted by the Microlab STAR resulted in the correct qualitative results for all replicates across multiple NxTAG RPP assay runs.

Conclusions: The Microlab STAR provides a considerable reduction in total NxTAG Respiratory Pathogen Panel assay time as well as hands-on time when >24 samples are run simultaneously and only a single easyMAG instrument is available. In addition, no changes to the NxTAG Respiratory Pathogen Panel assay's accuracy and precision were found when comparing the Microlab STAR extracted samples to traditional methods. Furthermore sample barcode-scanning, digital sample tracking, internal control liquid handling and direct pipetting from the original sample container eliminates points of user interaction that can lead to incorrect result generation and reporting.

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Abstract no: 15
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Comparison of viral and epidemiological profiles among hospitalized children with severe acute respiratory diseases in Beijing and Shanghai

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Objective: To character the viral and epidemiological profiles among hospitalized children with severe acute respiratory diseases (SARIs) in different areas of China.

Methods: Total 700 of NPA specimens, 259 from Beijing (North of China) and 441 from Shanghai (South of China), were collected from hospitalized children with acute respiratory diseases between May 2008 and March 2014. Multiple respiratory viruses were screened by validated PCR or real time RT-PCR and confirmed by sequencing, including Flu A/B, ADV, RSV, PIC (RV/EV), PIV1-3, HCoV (-OC43, HCoV-229E, HCoV-NL63, HCoV-HKU1), HBoV, hMPV. And the demographic data and distribution of viral infections were also analyzed.

Results: Total 547 from 700 samples were detected as positive for viral infection 78.1% (547/700). Among them, PIC (RV/EV) (34.0%) was the most detected, followed by RSV (28.3%), HBoV (19.1%), HCoVs (10.7%), ADV (13.7%), Flu A/B (8.9%), PIV 1-3 (7.9%)and hMPV (5.0%). PIC (RV/EV) and RSV were the most dominant etiological agents among hospitalized children with ARIs in both city of China. The prevalence of RSV, HCoVs, hMPV and co-infection was significant higher in Beijing than in Shanghai ($P < 0.05$). Different age and seasonal distribution of various viral infections between Beijing and Shanghai were also observed among hospitalized children with SARIs.

Conclusions: Different viral and epidemiological profiles existed between Beijing and Shanghai of China. The data provide a better understanding of the role of location and climate in the respiratory viral infection among hospitalized children with SARIs.

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Abstract no: 150
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The "Snotbarometer": Epidemiological data on respiratory infections

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Molecular detection of respiratory viruses was initiated in the Laboratory of Microbiology of OLVZ Aalst, Belgium, in 2003 with the detection of human metapneumovirus (hMPV) and respiratory syncytial virus (RSV). Since then, a constant elaboration of the portfolio was performed resulting in 8 multiplex in house real time PCR's that detect 22 respiratory pathogens including viruses (RSV, hMPV, adenovirus, bocavirus, para-influenzavirus (PIV) 1, 2, 3 and 4, Influenza A and B, coronaviruses, enterovirus and rhinovirus) and atypical bacteria (*M. pneumoniae, C. pneumoniae, B. pertussis, parapertussis* and *holmesii*). Samples are mainly obtained from our hospital but also from other hospitals from the Flanders region. On each respiratory sample for which molecular diagnostics for at least one of these pathogens is requested, the complete PCR panel of 22 pathogens is performed. This increases the accuracy of a specific diagnosis, and it also results in "local" epidemiological data. These data are translated into a graphic representation, called the "snotbarometer", which is made available for the hospital staff through the intranet, and on the website of the hospital. The "snotbarometer" consists of a weekly and a monthly report.

In the weekly report, the amount of positive samples for each pathogen separately is depicted in a graph and updated weekly. This presentation gives the physician an idea of the actually circulating pathogens, of the amount of samples analysed in the lab, and the percentage of samples positive for each pathogen.

In the monthly report a seasonal overview is given for the pathogens with epidemiological data available for multiple years, so one can start to extract the characteristic seasonal patterns. Examples are RSV, influenza A and B, PIV1, PIV2, PIV3 and PIV4. This year, Influenza B exceptionally preceded Influenza A which prolonged the influenza season. For other pathogens like adenovirus, bocavirus and *M. pneumoniae* the seasonality is less clear and one can observe a more fluctuating presence. Together, this information is very useful to predict the upcoming viruses.

Conclusion: Regional epidemiological data are powerful since they can give useful information to the physician, especially when a weekly follow-up is available.

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Abstract no: 181
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Molecular characterization of human parainfluenza virus type 3 (HPIV-3) among hospitalized patients from central Israel

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Human parainfluenza virus 3 (HPIV-3) is an enveloped, non-segmented, negative sense RNA virus that belongs to the *Paramyxoviridae* family. HPIV-3 is a common cause of bronchiolitis and pneumoniae in children less than 1 year of age and one of the leading causes of acute lower respiratory tract infections in children under five years of age. In Israel, the epidemiology of HPIV-3 infections is not well characterized.

In this study, epidemiology and molecular characterization of HPIV-3 was performed on patient samples collected between January 2012 and September 2015. Nasopharyngeal swabs ($N = 15{,}946$) were collected from hospitalized patients presenting with respiratory illness. Viral nucleic acid was extracted from patient sample using NucliSENS® easyMAG® (bioMérieux, France) and tested for the common human respiratory viruses (influenza viruses A and B, hMPV, adenovirus, RSV and HPIV-3) using validated real time PCR multiplex assays. Furthermore, molecular characterization of HPIV-3 complete HN gene (1722 bases) was performed after sequencing the complete HN gene. The Bayesian Markov chain Monte Carlo (MCMC) method was applied using a relaxed molecular clock, as implemented in the BEAST program (version 1.7.5). Trees were visualized and edited with the FigTree program (version 1.4.2) included in the BEAST software package.

Of the patient samples tested, 547 (3.43%) samples were positive for HPIV-3. Stratifying HPIV-3, by month revealed the virus major activity was during the winter and spring seasons. Not only that, but the majority of patients infected were children less than 1 year of age and elderly greater than 60 years of age. An increased HPIV-3 activity was seen in patients hospitalized in the oncology/transplants wards of the hospital. Of interest were patient's co-infections with HPIV-3 and other respiratory viruses. Of the 547 patient infected with HPIV-3, 99 (18.1%) patients were co-infected with other human respiratory viruses. Of which, adenovirus (6.6%) and RSV (6.4%) were the most common.

Molecular characterization of the complete HPIV-3 HN gene from 50 different patients infected throughout the study period revealed that the majority of the HPIV-3 strains circulating in Israel belonged to the C1b and C3a clades. These HPIV-3 clades were mainly seen in the America's and Saudi Arabia. In addition, one HPIV-3 isolate from the year 2012 did not match with any of the C1 clades, suggesting the possibility of being a new sub clade. HPIV-3 HN sequence analysis also revealed that the isolates characterized from Israel did not acquire the substitutions T193I and I567V in the HN gene suggesting that in patients with severe infection and where Zanamivir treatment is warranted, this antiviral can be used to help in managing the HPIV-3 infection.

This is the first comprehensive study that characterized HPIV-3 infections in Israel. The high co-infection rate of HPIV-3 and other common human patients mandates careful evaluation of the clinical presentation of infected patients and their prognosis. In addition, in depth evaluation of the clinical presentation of patients infected with the different HPIV-3 clades should be entertained.

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Abstract no: 182
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Prevalence and genetic characterization of enterovirus D68 among children with severe acute respiratory infection in China

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To understand the prevalence and molecular typing of enterovirus D68 among children with severe acute respiratory infection (SARI) in Beijing and Shanghai, 385 respiratory samples were collected from in Beijing during 2008–2010, and 441 respiratory samples were collected in Shanghai city between 2013 and 2014. All the samples were used for the screening of EV-D68 by nest RT-PCR and sequencing, then EV-D68-positive samples were used for the complete genome sequencing through overlapping PCR. All available EV-D68 full-length genomes collected from GenBank were used for phylogenetic analysis and comparison of EV-D68 types prevalent in China and America. One (0.4%) from 385 respiratory samples in Beijing was positive for EV-D68, and 4 (0.9%)among the 441 samples from Shanghai were positive for EV-D68. Phylogenetic analysis of full length genome indicated that the EV-D68 prevalent in Beijing (BJ24) belong to Clade A2 and Clade B2, different from the American popular strains (Clade A1, Clade B1, Clade B4 and Clade B5). Partial sequence analysis declared phylogenetic conflict among different gene sequences. We concluded that the prevalence rate of EV-D68 among SARI Children in Beijing and Shanghai currently was lower (5/700; <1%), and the EV-D68 genotype prevalent in China and America belong to different clusters. Partial sequence analysis indicated that intratypic recombinant events may occur in EV-D68 prevalent in China.

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Abstract no: 186
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Replication and immune response in HAE of HCoV-HKU1 isolate from a pediatric patient with severe acute respiratory infection

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Human coronavirus HKU1 (HCoV-HKU1), a fastidious cultured β-coronavirus, was associated with acute respiratory infection in the aged and children. Human airway epithelium cells (HAE) provide the first line of defense in the respiratory tract and are the main target of HCoV-HKU1. However, little attention has been devoted to immune response of HAE induced by HCoV-HKU1, maybe due to its fastidious culturing. Here, we isolated a novel strain of HCoV-HKU1 (BJ-01) from a pediatric patient with severe acute respiratory infection (SARI) and propagated on HAE. This stain of virus owned the typical morphology of coronavirus with the diameter of 120–130 nm. The genome HCoV-HKU1 BJ-01 is conserved during serially passage on HAE cells. Comparing viral genome of the early passage (P3, Gene accession No KT779555) with that of late passage (P9, Gene accession No KT779556), there were only two amino acid substitutions on ORF1b (T2346C) and S (G23216T) glycoprotein. We further investigate how the immune response in HAE to HCoV-HKU1 infection using Quantibody® Human Cytokine Antibody Arrays (RayBiotech, Inc.). We found 31 cytokines increased and 55 cytokines decreased more than 2 folds in the 640 detected cytokines. These cytokines can be divided into different groups: Chemokines (CCL4, CCL13, CCL15, CCL16, CCL24, CCL26, CXCL13, XCL1), Hematopoietins (IL23R, TSLP, PRL, GHR), PDGF family (PDGFC, KITLG), IL-10 family (IL20RA), IL-1 family (IL1R1), TNF family (SF11B, LTA, SF1B), TGF-β family (BMP7, BMPR1B), which mainly affect Chemokine/NF-KAPPA B/PI3K-AKT/JAK-STAT signaling pathway on HAE cells. This work was the first report on immune response in HAE of a novel HCoV-HKU1 strain (HCoV-HKU1 BJ01) from a pediatric patient with SARI.

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Abstract no: 19
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Development of an external quality assessment panel for the molecular detection of respiratory viruses

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Background: Respiratory virus infections occur commonly and are responsible for a significant amount of morbidity worldwide. In the developed world respiratory viruses are responsible for a considerable amount of morbidity which has a significant economic impact. Mortality rates however are low. In contrast, in developing countries, viruses are responsible for approximately 20–30% of respiratory deaths in children. The spectrum of disease ranges from upper respiratory tract infections such as common colds to infections of the lower respiratory tract manifesting as bronchiolitis or pneumonia.

Respiratory Syncytial Virus (RSV) is the most common cause of lower respiratory tract infection in infants and children worldwide. Most frequent types of influenza viruses that affect individuals are Influenza A and B, however rhinoviruses are the common cause of coughs and colds during winter with a peak season in the UK to be between January and March.

The elderly and infant population tend to be the most susceptible to these viral illnesses. The availability of an External Quality Assessment (EQA) panel for the Molecular Detection of Respiratory viruses is crucial for providing objective evidence in the quality of testing with a request.

Materials and methods: A pilot distribution, containing 9 specimens was sent out to 23 participants. Three simulated throat swabs and six simulated freeze-dried nasopharyngeal aspirate material (Specimen numbers were 3642–3650) were dispatched for testing for respiratory pathogens using molecular methods. The swab specimens were distributed in a Phosphate Buffer Solution (PBS) based solution with a different cell line added in each of them. The freeze dried specimens were distributed in a sucrose based matrix. Simulated specimens were positive for Parainfluenza virus type one (PIV-1), Adenovirus type 2 (AdV-2) and Influenza B (FluB).

Results: Out of 23 participants, 21 returned their results. Only one laboratory detected PIV-1 in specimen 3642. PIV-1 was correctly reported for specimen 3643 mainly by those laboratories who have used in-house real-time multiplex/single target PCR assays. RespiFinder was the only commercially available multiplex real-time assay that was able to detect PIV-1 in specimen 3643.

Adenovirus type 2 was successfully detected for specimens 3644, 3645 and 3649 by all those laboratories tested 100% for adenoviruses. Performance was excellent for Flu-B with 100% of

participants reporting the correct virus for specimen 3646, 3647 and 3650. A new pilot is due to be sent out in June 2016 and it will contain 6 freeze–dried samples with the same matrix as previously used.

Conclusion: This study established that there is a need in offering a scheme for the molecular detection of the most important Respiratory Viruses. The new scheme is expected to be available from April 2017 and is available for laboratories that performing molecular testing for respiratory pathogens.

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Abstract no: 198
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Genotyping and epidemiology analysis of human rhinovirus among children with severe acute respiratory infection in Shanghai, China 2013–2014

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Background: Human rhinovirus (HRV) belongs to the Enterovirus genus and Picornaviridae family, which has been classified into three species, HRV-A to HRV-C. HRV is a common cause of mild acute respiratory infection (ARI) in children. However, in recent years, HRV has been identified as an etiological agent of severe acute respiratory infection (SARI) C such as pneumonia, bronchiolitis and asthma, especially HRV-C discovered in 2006. In China, HRV species, epidemiological data and clinical profiles are limited on HRV infection in children with SARI.

Objectives: To investigate the epidemiological characteristics, clinical features and genotype diversity of human rhinoviruses from children with severe acute respiratory infections.

Study design: 441 nasopharyngeal aspirates were collected from children with SARI in Shanghai from 2013 to 2014. Nested RT-PCR was employed for preliminary HRV detection and phylogenetic analysis on VP4/VP2 region was used to further characterize the viruses. The clinical and epidemiological characteristics of the patients with HRV infection were analyzed.

Results: HRV was detected in 132 (29.9%) of the 441 specimens: HRV-A in 70 (56%), HRV-B in 10 (7.6%), HRV-C in 31 (23.5%) and HRV untyped in 21 (18.9%). HRV-A to HRV-C detections peaked in September, August and October, corresponding with autumn, respectively. A higher detection frequency of HRV-A and HRV-C occurred in the <5 years age groups. Cough, fever and wheezing were the most common symptoms and pneumonia was the most common diagnosis in patients with HRV infection. Overall there were no significant differences in the clinical symptom and diagnosis between the patients infected with HRV-A and HRV-C. HRV-C is recently discovered species and may be associated with SARI, asthma and wheezing. Phylogenetic analysis showed that HRV-C strains in present study belonged to C3, C6, C13, C15, C16, C18, C24, C27, C30, C37 and C39 genotypes especially, and C6, C16 and C24 were the most frequently genotypes, which were different from the reports from Japan, Paraguay and Hong Kong China. In addition, 53 (40.2%) HRV positive patients were co-infected with other respiratory viruses, among which the most HRV-associated co-infections involved human bocavirus (HBoV), adenovirus (ADV) and human parainfluenza virus (PIV).

Conclusion: Our study showed that HRV was frequently present in children with SARI in Shanghai, China. The clinical symptom and diagnosis of patients infected with HRV-A were similar with HRV-C species. 11genotypes for HRV-C were found and C6, C16 and C24 genotypes were the most common.

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Abstract no: 204
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RSV surveillance in Sweden – How can we improve it?

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Introduction: National RSV surveillance in Sweden aims to guide clinicians through the RSV season and help them to optimize the timing of RSV immunoprophylaxis, in order to prevent serious cases of RSV among infants. Estimating the burden of RSV infection will likely become an important aim of surveillance as vaccines approach the commercial market. We evaluated how RSV diagnostics is currently performed in Sweden and the usefulness of RSV surveillance to clinicians.

Methods: Clinical microbiology laboratories ($n = 24$) and clinics ($n = 5$) are invited to report all laboratory-confirmed RSV infections to the Public Health Agency of Sweden. These data are analysed and results published weekly. The first questionnaire on methods was sent to all reporting units, and the second on usefulness of RSV surveillance to readers of the weekly report. Swedish Quality Control Program (EQUALIS) data on the performance of laboratory testing were also analysed.

Results: 23 laboratories and four clinics responded to the first questionnaire, and 77 readers to the second. All except two laboratories use commercial and/or in-house PCR (15 and 7 laboratories, respectively). RSV PCR is also performed in seven clinical chemistry laboratories; none participate in EQUALIS or RSV surveillance. Chromatographic antigen detection (referred as near-patient test, NPT) was reported to be used by three laboratories and eight clinics; three of these clinics do not currently participate RSV surveillance. NPT-results obtained only in two laboratories and one clinic are confirmed by another assay. RSV-NPT sensitivity was 0.23 based EQUALIS panel 2015. Reporting took 15 min/week (4–60 min); nine laboratories found it time-consuming. Most clinical readers use the published RSV data (38/45), either to guide timing of RSV immunoprophylaxis ($n = 14$), as staff information ($n = 10$) or for hospital preparedness ($n = 14$).

Discussion: RSV surveillance data is widely used by clinicians, but a need for a faster reporting platform was identified. Furthermore, the importance of confirming RSV-NPT results should be highlighted. Clinical chemistry laboratories and clinics performing RSV-NPT will be encouraged to participate in EQUALIS and RSV surveillance. These measures will improve Swedish RSV surveillance and awareness of RSV in the pre-vaccination era.

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Abstract no: 221
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Microarray-based molecular detection of viral pathogens associated with respiratory infections

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Objective: Acute respiratory infections are one of the most common infectious diseases. Clinical course of acute respiratory infections in young children and the elderly may be serious and life-threatening. It has been emphasized that 20–60% of etiologic agents are viruses. Respiratory syncytial virus (RSV) is the most common viral pathogen in children. On the other hand, influenza virus is the most commonly identified agent in adults. The aim of this study is to identify the viral pathogens in hospitalized patients with respiratory tract infection by using multiplex PCR method.

Methods: Nasopharyngeal swab samples obtained from hospitalized patients with acute respiratory infections were enrolled between January 2013 and December 2015. The identification of influenza virus type A and B, human rhinovirus (HRV), respiratory syncytial virus A and B (RSV A-B), parainfluenza virus type 1, 2, 3, and 4, adenovirus, bocavirus, human coronavirus, human enterovirus and human metapneumovirus (HMPV) in nasopharyngeal samples was investigated by using CLART®Pneumovir kit based on clinical array technology (Genomica, Spain).

Results: Of the 1290 patients included, 1110 (86%) were children and 180 (14%) were adults. The number of samples in which only one virus was identified was 600. In additional 150 specimens, co-infections of multiple viruses were detected. The total of positive samples was 750 (58%). The majority of these positive specimens were children's samples (694 versus 56). RSV was the most common viral agent (35%) followed by HRV (13%), and influenza (10%). The rate of co-existence of viral pathogens was 20%. The multiplex pcr results were shown in Table 1.

Conclusion: While RSV was the most common viral pathogen detected inrespiratory infections, other emerging agents, such as human metapneumovirus, bocavirus, and HRV were detected in considerably high rates, suggesting these emerging agents should not be underestimated in the etiology of respiratory infections. In conclusion, in this study, it was shown that microarray-based multiplex PCR method is an easy, rapid, and sensitive diagnostic tool for diagnosis of viral respiratory infections and that utility of this method makes it essential among routine diagnostic tools in clinical microbiology laboratories.

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Table 1
Distribution of respiratory virusus in positive samples.

Viral agents	% (*n*)
RSV	35 (266)
HRV	13 (94)
Influenza type A–B	10 (78)
Bocavirus	6 (45)
Parainfluenza virus	6 (44)
Metapneumovirus	5 (35)
Adenovirus	4 (30)
Enterovirus	0.7 (6)
Coronavirus	0.3 (2)
Co-existence	20 (150)
Total	100 (750)

Abstract no: 228
Presentation at ESCV 2016: Poster 192

Detection of influenza viruses from patients in university hospital

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Objective: Influenza epidemics occur almost every winter and are associated with considerable morbidity, mortality and economical lost. All age groups are susceptible, but increasing age, certain chronic medical conditions, immundeficiency, pregnancy increase the risk of complications and death. Influenzae A and B are responsible for annual epidemics. Quick diagnosis of influenza by laboratory methods are important for treatment of flu and prevention of epidemics.

The aim of this study is to detect prevalence of influenza virus and its subtypes in patients who admitted to University Hospital in Konya province with flu like symptoms.

Material and methods: Nasal swap samples of patients with flu like symptoms who admitted to various clinics in Necmettin Erbakan University Meram Medical Faculty in Konya; Turkey between January 1, 2013 and May 25, 2016 were tested for Influenza A and Influenza B by CLART® PneumoVir (Genomica, Madrid, Spain) micraarray method and Seeplex® RV12 ACE Detection multiplex PCR (Seegene, S. Korea).

Results: Results of total 2041 samples are analyzed retrospectively. 258 (12.6%) samples were positive for influenza virus. 97 (4.7%) were found to be positive for influenza A and 161 (7.8%) for influenza B.

Conclusion: Influenza B was found to be the predominant subtype in patients who admitted to hospital with flu-like symptoms in Konya province. In our study January to March were the months with the highest percentage of testing positive for influenza virus infection. Influenza virus can be detected from respiratory samples with high sensitivity by molecular methods such as microarray method and multiplex PCR. Observing seasonal activity and epidemic strains and starting early treatment and taking isolation measures are important for preventing rapid spread and progression of virus and has critical role for public health.

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Abstract no: 233
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An overview of human parainfluenza virus 1-4 infections in northeastern Slovenia based on molecular detection

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Introduction: Human Parainfluenza viruses (HPIVs) are one of the most common causes of upper and lower respiratory tract illnesses, and an important cause of hospitalizations among children under 5 years old. There are currently no published data on HPIV infections in Slovenia.

Methods: In this retrospective study we examined a total of 2637 samples (nasopharyngeal, nose and throat swabs). They were taken from patients with symptoms of respiratory infection admitted to University Medical Centre Maribor during the years of 2014 and 2015. HPIV RNAs were detected with a commercial automated multiplex PCR system (FilmArray, Biofire).

Results: Out of 2637 samples, 173 (6.56%) tested positive for HPIVs. Nearly half of the HPIV-positive patients were infected with HPIV-3 (49.71%, 86), followed by HPIV-4 (21.39%, 37), HPIV-1 (16.76%, 29) and HPIV-2 (15.03%, 26), respectively. Most frequently identified type was HPIV-3, with regular activity throughout 2014 and 2015, including a substantial increase in both autumn-winter seasons with peaks in November of 2014 and 2015. It was also the predominant HPIV type represented in summer months of both years alongside minute occurrences of type 2 and 4. An apparent outbreak of HPIV-4 infections starting in summer, and progressing in autumn of 2015 with a peak in September, was observed. At the same time, HPIV-3 and HPIV-2 were in decline. Also, type 1 and 3 started to increase as HPIV-4 decreased. Type 2 was completely absent in spring 2014 but had a slight peak in October 2014 and was subsequently present in smaller numbers for the rest of 2015. The median age of HPIV-tested patients was 5, and ranged from less than a year to 96 years old. The majority (82.08%, 142) of infected patients were children under the age of 5. Among the elderly (>65 years old) 12.75% (13/102) tested positive for one of HPIVs, the oldest being 87 years old. The male to female ratio of patients infected with HPIV was 1:1. HPIV was detected as the only cause of infection in 60.11% (107) of cases and 5 of them tested positive for two types of HPIV. In forty-eight (26.97%) HPIV-positive samples one co-infection with other respiratory pathogen was detected, 18 (10.11%) had two co-infections and 5 (2.81%) had three or more co-infections. The prevalent (50.00%) pathogen of co-infection was rhinovirus, followed by adenovirus in 18.00%, enterovirus in 12.00% and respiratory syncytial virus in 10.00% of samples. Coronavirus (HKU1 and OC43), *Mycoplasma pneumoniae*, human metapneumovirus and *Bordetella pertussis* accounted for the remaining 10.00%.

Conclusions: Overall, the analysed data suggests HPIV-3 as the most prevalent type of HPIV infections in NE Slovenia. Both HPIV-2 and HPIV-3 showed continual presence in the studied 2-year period with the latter greatly outnumbered the former. A similar biennial distribution pattern for HPIV-1 and HPIV-4 was noted, which could mean that they tend to occur in odd-numbered years. We also observed an epidemic of HPIV-4 which is rarely reported in literature. From previously published reports it appears that seasonal trends vary in different parts of the world and that the distribution of HPIV types is also affected by environmental conditions. Additional data from following years is needed for a more clear understanding of HPIV seasonal trends and interactions between all four types in NE Slovenia.

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Seasonality of respiratory syncytial virus infection in the EU/EEA, 2010–2016

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Background: Respiratory syncytial virus (RSV) is considered the most common pathogen causing severe lower respiratory tract infections among infants and children. RSV vaccine candidates are in development and the World Health Organization is preparing global RSV surveillance to estimate the impact of future RSV vaccines. One of the surveillance objectives is to monitor RSV seasonality and intensity. A subset of EU/EEA Member States (MS) are already testing clinical specimens for influenza and RSV as part of their routine influenza surveillance. In this study, we are describing the seasonality of RSV infection in these countries.

Methods: We performed a retrospective descriptive study of laboratory-confirmed RSV detections reported weekly through the European Influenza Surveillance Network based on influenza-like illness (ILI) or acute respiratory infection case definitions between weeks 40/2010 and 14/2016. We compared findings between systematically sampled primary-care-based sentinel specimens tested according to a standard protocol and convenience sampled primary and hospital-care-based non-sentinel specimens. We also studied the correlation between the median week of peak RSV detections and the latitude of each reporting country's capital by Pearson's correlation. RSV seasons were defined as the number of weeks when detections exceeded 5% of total detections per season per country.

Results: Seventeen MS reported RSV detections during the study period: seven MS reported 4399 sentinel detections and fifteen MS reported 156,698 non-sentinel detections. Two MS contributed 60% of sentinel and 61% of non-sentinel detections. Seasonality was observed within both surveillance systems. The median length of RSV season estimated based on sentinel and non-sentinel surveillance was 11 (with country range 6–28) and 10 (range 6–18) weeks, respectively. The median peak week for sentinel detections was week 6 (range 48–18), and for non-sentinel detections week 5 (range 49–17). RSV was detected by non-sentinel surveillance throughout the year but in sentinel system only during weeks 45–13 with consistent reporting. RSV detections peaked later with increasing latitude ($r = 0.41$ for sentinel and 0.46 for non-sentinel).

Conclusions: RSV detections in 17EU/EEA MS followed a seasonal pattern, peaking regularly early February and lasting around 10 weeks. Our data confirm the moderate correlation between the timing of the epidemic peak and increasing latitude that has been shown earlier. Our study suggests that RSV seasonality can be assessed through both sentinel and non-sentinel influenza surveillance systems but more sensitively in the latter one. Overall, the number of sentinel RSV detections were vastly lower compared to non-sentinel specimens which is a reflection of different surveillance systems and number of participating countries. We do not have RSV-specific denominator data and can therefore not calculate proportions. Further limitations of the data include that large detection volumes originate from only two MS. Despite the limitations, this study supports the use of influenza surveillance systems for monitoring RSV seasonality with consideration to adjust the ILI case definition to establish an RSV-specific surveillance system. Further-

more, for monitoring of intensity, RSV-specific denominators are needed.

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Herpes Simplex 1-2 in broncho alveolar fluid: A 5 years retrospective study

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Aim: Respiratory viruses are very often detected in pneumonia thanks to uniplex and multiplex real-time PCR techniques. Moreover viruses that are not primilarly the cause of respiratory infections may also be detected such as HSV 1-2, varicella zoster virus and cytomegalovirus.

The aim of our study was to analyze the prevalence of other respiratory infections among broncho-alveolar lavages (BAL) positive for HSV 1-2. The clinical outcome of patients according to the anti HSV 1-2 treatment was also analyzed.

Material and methods: Data from hospitalized patients suffering from serious respiratory symptoms and whose broncho-alveolar lavages were positive for HSV 1-2 by an in-house real-time PCR were analyzed.

Nucleic acids (NA) had been extracted with the MagNA Pure 96 DNA and Viral NA Small Volume kit® on the MagNA Pure 96™ instrument (Roche Molecular Diagnostics, Meylan, France).

Samples had been tested for 16 respiratory viruses (influenza A and B, parainfluenza 1–4, respiratory syncytial viruses A and B, human metapneumovirus, coronaviruses 229E, OC43 and NL63, rhinoviruses, enteroviruses, bocaviruses and adenoviruses) with a multiplex RT-PCR (Anyplex™ II RV16 Detection®, Seegene) on the CFX96™ Real-Time System (Biorad diagnostics).

NA had also been tested for cytomegalovirus, varicella zoster virus employing a monoplex in-house PCR on the Light Cycler or the Light Cycler 480™ (Roche Molecular Diagnostics, Meylan, France).

Data were analyzed on StataTM software (StataCorp, Texas) using the exact Fisher test.

Results: Between 2011 and 2015, 122 (73 males) patients attending an intensive care unit in the Toulouse University Hospital were selected with a HSV 1-2 positive result (mean age 62; 24–86). 119 samples were HSV-1 and three were HSV-2.

117 had been tested with the Anyplex™ II RV16 Detection Seegene®. All the viruses of the panel had been detected except parainfluenza 1, 2 and 4, human metapneumovirus and bocavirus; Influenza viruses were the most detected ($n = 13$), followed by rhinovirus ($n = 6$), respiratory syncytial virus ($n = 5$) and adenovirus ($n = 4$). 117 samples had been tested for cytomegalovirus (26 positive), and 90 for varicella zoster virus (negative).

28 among the 78 samples that tested positive for HSV 1-2 during the winter season (November to April) were also positive for another respiratory virus. During the summer season (May to October) 44 samples tested positive for HSV 1-2 with only 4 in coinfection ($p < 0.005$).

The mortality rate did not differ between the HSV 1-2 positive patients treated with acyclovir or valacyclovir ($n = 57$) and those who were not ($p = $ ns).

Conclusions: Our results indicate that HSV 1-2 infection is frequent among patients hospitalized in intensive care unit. During the winter season this infection is linked to other respiratory viruses.

The apparent clinical inefficiency of anti HSV 1-2 treatment indicates that the presence of the virus is more a witness of a clinically poor condition rather than a cause of it.

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Abstract no: 255
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Three years (2013–2016) of human respiratory syncytial virus surveillance at a tertiary hospital in Catalonia, Spain

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Background: Human respiratory syncytial virus (HRSV) is the most common respiratory pathogen and the main cause of lower respiratory tract infections among infants and young children. Its genome is a lineal single-stranded negative-sense RNA of approximately 15 kb that contains 10 genes encoding 11 proteins. The G glycoprotein in the viral envelope plays an essential role in the virus attachment. Antigenic and genetic differences in this protein lead classify HRSV into two different groups, HRSV-A and HRSV-B. Furthermore, based on the hypervariable region 2 (HVR-2) located in the C-terminal domain of the G protein several genotypes have been described. Selection pressure drives G protein to continuously evolve, resulting in the likely replacement of predominant genotype season by season. In the present study the epidemiology of HRSV viruses detected in respiratory specimens from patients attended at the Hospital Universitari Vall d'Hebron in Barcelona (Spain) during three consecutive years (from 2013 to 2016) has been described.

Material and methods: From October 2013 (week 40) to March 2016 (week 20) respiratory specimens from patients were collected for laboratory confirmation of respiratory virus infection using immunochromatography (Binax Now RSV Card, Allere Scarborough Inc, USA), immunofluorescence (D^3 *Ultra 8*™ DFA Respiratory Virus Screening & Identification Kit, Diagnostic HYBRIDS, USA) or real-time multiplex RT-PCR (Anyplex II RV16 Detection Kit, Seegene, Korea) assays. A nucleoprotein-specific real time RT-PCR was performed to determine HRSV group. In addition, phylogenetic analyses and molecular characterizations were carried out using MEGAv5.2 software based on the HVR-2 sequence from a representing sampling of HRSV per week.

Results: A total of 16552 specimens were collected, of which 1324 (8.3%) were positive for HRSV. The virus showed a seasonal pattern of circulation, previous to influenza annual epidemics, with a maximum detection rates in the weeks 52 or 53 in all three seasons. Viruses belonging to both HRSV groups were detected: HRSV-A (662; 50%), HRSV-B (579, 44%), HRSV-A/B co-infection (8; <1%), and 75 (6%) remained unsubtyped. There was an alternation in the predominance of HRSV group by season; while HRSV-B was predominant during the first two seasons, HRSV-A became it during the third. Based on HVR-2 phylogenetic analyses, HRSV-A viruses belonged to ON1 genotype (153; 99%), but 2 (1%) to NA1.

In addition, HRSV-B viruses belonged to BA9 (153; 83%), BA10 (9; 5%) and an undefined BA (1; 1%). Nevertheless, 21 (11%) sequences from 2014 to 2015 season, closely related to the BA9 genotype, clustered together with a bootstrap value of 100% showing a p-distance between members of 0.006 and an average divergence within group of 0.004. Therefore, according to the criterion used by Venter et al. (J Gen Virol 2001; 82 (9): 2117-24), they might belong to a novel genotype (purposed name as BA13 in this study). However, viruses belonging to this new genotype were not found during the subsequent 2015–2016 season.

Discussion and conclusions: Co-circulation of both HRSV groups has been reported during the three seasons. An alternation of the predominant HRSV group was shown during these three consecutive seasons. Although several genotypes were reported, the most of viruses belong to ON1 (HRSV-A) and BA9 (HRSV-B). The present study reports recent valuable data about the genetic diversity of circulating HRSV in the Southern Europe.

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Evaluation of interferon lambda 4 nucleotide polymorphism in infants suffering from bronchiolitis

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The clinical spectrum of respiratory syncytial virus (RSV)-associated bronchiolitis in infants is variable, ranging from a mild disease to a severe respiratory distress causing hospitalization. It is acquired that immune response and genetic heterogeneity of the host, together with well-known viral risk factors, contribute to RSV disease severity. Recent studies showed the importance of interferon (IFN) Lambda in the protection against RSV and other respiratory viruses. Our previous studies in bronchiolitis patients demonstrated higher mRNA levels of IFN Lambdas and of IFN-stimulated genes in RSV-positive infants than in infants with HRV infection.

Recently, it was shown that the novel ss469415590 SNP is more strongly associated with spontaneous HCV clearance and treatment-induced response than the IFN-lambda 3/IL28B SNP rs12979860. The ss469415590 SNP is a di-nucleotide mutant (TT > ΔG) located in the region upstream IL28B gene; the unfavorable ΔG allele is a frameshift variant creating the gene encoding a functional protein designated IFN-lambda 4 (IFNL4).

Given the importance of the IFN lambda in respiratory infections, we sought to evaluate whether IFNL4 SNP could be associated with bronchiolitis severity. Hence, infants admitted to the Paediatric Department of Umberto I University Hospital, with a clinical diagnosis of bronchiolitis, were tested for ss469415590 SNP. Bronchiolitis severity was assessed with a score, based on respiratory rate, arterial oxygen saturation, presence of retractions and ability to feed (score range 0–8). For each sample, detection of 14 major respiratory viruses was performed and 122 samples positive only to RSV were selected for this preliminary study. DNA for the haplotype analysis was obtained from a buccal swab, when available or from archivial cell pellets from respiratory samples. TT/ΔG genotyping was performed with the "StepOne Real-Time PCR System" method, using primers specific for the amplification of the polymorphic sequence and two TaqMan-MGB probes specific for each allele (*Express program and Genotyping assay service* Applied Biosystem).

The presence of at least one ΔG allele (homo- or heterozygous) was significantly associated with overall disease severity (severity score 5–8), and related clinical parameters (but not with length of hospital stay, age or weight at hospital admission, weight at birth or gestational age, number of blood cells). Our previous study showed that infants carrying IL28B rs12979860 TT allele, that is in strong/moderate linkage disequilibrium with the IFNL4 ΔG allele, had lower age at hospital admission, but did not suffer from a more severe bronchiolitis course. However, that study did not examine determinants of bronchiolitis severity in the RSV-infected children separately, because of a smaller number of samples.

The present data suggest the importance of detecting IFNL4 SNPs in a larger group of infants affected with bronchiolitis. Further studies are needed also to understand the protective or detrimental effects of IFNL4 production during respiratory virus infections.

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Patterns of respiratory pathogen nasal colonization in the first year of life in healthy infants and infants with cystic fibrosis

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Introduction and study aims: Respiratory infections are known to play a major role in morbidity and mortality, especially in early childhood and infancy. A number of studies have investigated pathogen colonization in otherwise healthy infants using PCR analysis of nasal swab material as an established diagnostic method. However, little is known about pathogen colonization in infants with chronic respiratory diseases like cystic fibrosis (CF). The aims of our study were: (1) to investigate feasibility and quality of parental collected nasal swab material for respiratory diagnostics; (2) to analyze possible differences in viral and atypical pathogen (*Chlamydophila pnumoniae*, *Mycoplasma pneumoniae*) colonization in healthy infants compared to infants with CF.

Methods: 31 infants with CF and 32 unselected healthy infants were included in this prospective longitudinal study spanning the first year of life. Biweekly nasal FLOQSwabs™ ($n = 1398$) placed in UTM-RT™ (Copan, Italia) were collected by parents after

instruction by study nurses. Nucleic acids were extracted from 400 μl of sample using NucliSENS easyMAG (bioMérieux, Marcy l'Etoile, France). RNA and DNA were eluted in 110 μl and analyzed by real-time PCR using a combination of 7 duplex Respiratory Multi Well System r-gene™ assays (influenza A/B, RSV/hMPV, Rhino&EV/cell control, ADV/HBoV, HCoV/HPIV1-4, Chla/Myco and PeV) (Argene/bioMerieux,Marcy l'Etoile, France), according to the manufacturer's instructions. Sample quality was evaluated using a HPRT1 cellular gene control (CC) assay (included in the Rhino&EV assay) that evaluated the quantity of human epithelial cells present in the sample.

Results: CC was positive in 93% of the samples (1294/1398) and among those, 98% and 87% were positive in healthy and CF infant groups, respectively. Semiquantitative analysis of positive CCs and virus positive samples did not differ between the two groups. Analyses of the CT values (with and without inclusion of low quality swabs) did not demonstrate any differences between both study groups. Rates of viral colonization were similar in healthy and CF infants (43% and 42%, respectively), however there were clear differences in PIV colonization (11% and 6%, respectively; $p = 0.038$) and bocavirus colonization (6% and 23%, respectively; $p < 0.001$). HRV was the most frequent virus identified in healthy and CF infants (57% and 46%, respectively).

Conclusion: This study demonstrated that parental collection of nasal swabs from healthy and CF infants provided easy and adequate material for testing. Although the number of low quality swabs was slightly higher in the CF group, sensitivity analysis showed that this did not bias the results. Possible reasons for lower quality may be more careful swabbing by parents of infants with CF or viscous mucus in the nose. Interestingly, while viral colonization in general was similar in healthy and CF infants, there were clear differences in viral species, a finding of importance for future treatment options and understanding disease development.

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Abstract no: 269
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Genotyping of rhinoviruses in children and adults during 2014–2016

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Background and objective: Rhinoviruses (RV) are major causative agents of acute respiratory tract infections in children and adults. Rhinoviruses can be divided into three species (RV-A, RV-B, and RV-C) including many different viral types on the basis of their genetic characteristics. The objective of the study was to determine the distribution of RV species in respiratory infections between January 2014 and 2016 in Aydın, Turkey, and to investigate the relationship between the RV species and clinical symptoms.

Material and methods: Between January 2014 and 2016, a total 127 nasopharyngeal swabs samples were collected from patients submitted to Adnan Menderes University Hospital in Aydın, Turkey. The screening of respiratory viruses (HAdV, FLUAV, FLUBV, RV, HCoV- 229E/NL63, HCoV-OC43, HMPV, HPIV-1, HPIV-2, HPIV-3, HPIV-4, HEV, HRSVA, HRSV-B and HBoV) was performed with two commercial multiplex PCR-based method (*Anyplex* II *RV16, Seegene*, South Korea and FTD Respiratory pathogens 21 plus, Luxemburg). The RV-positive samples were sequenced in the VP4/VP2 regions.

Results: Of the 127 samples, 96 (75.5%) were positive (50 children, 46 adults). The median age for children was 24 months (6.19–72) and the mean age for adults was 57.63 ± 15.68. From a total of 96 rhinovirus-positive samples, 65 (33 children and 32 adults) were sequenced in the VP4/VP2 regions. Twenty-eight (43.07%) samples were identified as RV-A and 7 (10.76%) as RV-B, and 28 (43.07%) samples belonging to the RV-C species. EV-D68 was detected in only one adult patient.

Conclusion: To our information, this is the first study about RV genotyping in children and adult patients in Turkey. We have detected RV-A and RV-C being the most prevalent species, and HRV-B trailing behind No significant relationship between the RV species and clinical symptoms was observed.

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A cost-efficient solution: Reagent comparison guide for neuraminidase inhibition assay

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Background: The neuraminidase (NA) inhibitors (NAIs) encompass three FDA-approved compounds: oseltamivir (Tamiflu®), zanamivir (Relenza®) and peramivir (Rapivab®); along with laninamivir (Inavir®) approved in Japan [Takashita et al., 2015]. The potential for emergence and spread of NAI-resistant viruses and the limited therapeutic options available reinforce the importance of NAI susceptibility surveillance [Okomo-Adhiambo et al., 2014].

Phenotypic profiling of influenza virus (IV) susceptibility to NAIs tested by neuraminidase inhibition assay (NIA) has been the recommended methodology to use, allowing to determine the concentration of NAI required to inhibit 50% of the virus NA activity (IC50) [WHO, 2012]. The central reagents used in this assay, specifically the MUNANA substrate and the antiviral drug can be obtained from multiples pharmaceutical companies at a different price. Consequently, there is the need of practical guidance regarding the chosen reagent suppliers, which may have an effective outcome on the reported inter-laboratory results. In this context, the evaluation of available reagents is essential to determine the efficacy of the alternative sources for MUNANA and NAIs and possibly provide a cost-efficient alternative solution.

This study aimed to compare available alternative reagents for NA activity assay (NAA) and NIA and to assess the phenotypic susceptibility profiles of IV from different (sub)types to the new NAIs laninamivir and peramivir.

Methods: Twelve IV were selected for study: 6 virus isolates from a reference panel (isirv-Antiviral Group); and 6 clinical specimens positive for influenza virus. Phenotypic assay was performed using an in-house MUNANA-based IC50 fluorescence assay [HPA, 2006].

NAA was performed using the MUNANA substrate obtained from both Biosynth AG and Sigma-Aldrich companies. The NIA was conducted for both MUNANA brands to assess the susceptibilities of all IV to oseltamivir and zanamivir.

Additionally, NIA was performed for each IV strain with alternative sources of NAIs: oseltamivir – F. Hoffmann-La Roche Ltd vs. oseltamivir – Sequoia Research Products Ltd (SRP); zanamivir – GlaxoSmithKline vs. zanamivir – SRP; laninamivir – Daiichi Sankyo vs. laninamivir – SRP and peramivir – BioCryst Pharmaceuticals.

Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference post-hoc test ($\alpha = 0.05$) using R.

Results: No statistically significant difference was established for the IC50 means of each pair of commercially available reagents.

MUNANA from both companies performed similarly in determining NA activity of the selected IV and exhibited the same profile in determining the IC50 mean values for oseltamivir and zanamivir.

IC50 values of the selected IV determined for oseltamivir, zanamivir and laninamivir exhibited the same potency for the different sources of NAIs.

Conclusions: MUNANA substrate compound and NAIs previously available from a single supplier can now be purchased from other chemical companies and at a significantly low price. Given the limited resources of research and public health funding, preference for alternative suppliers might be translated into cost savings or low bureaucratic nuisance. This strategy may maximize funding resources and allow researchers to divert more funds to targeted research goals. Laboratories are encouraged to consider these cost-efficient alternative suppliers as a reliable solution.

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Evaluation of point of care testing platform (ePLEX) for respiratory viral diagnosis

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Background: There is an increasing demand on laboratories to deliver respiratory viral diagnosis by molecular methods. Different strategies are explored which include – point of care testing which is simple, requires minimal hands on time, is fast and can be done in ward areas and not in centralised laboratories. Tests considered need to be shown to have good performance.

GenMark Diagnostics Inc. (Carlsbad, USA) has developed a respiratory panel assay (RP) for the ePlex system detecting 26 microbes, including 22 virus and 4 (atypical) bacteria in 90 min.

The RP cartridge contains all reagents required to run the RP Panel assay. Lysis and nucleic acid extraction, PCR amplification and hybridization-based electrochemical detection occur inside the cartridge, reducing the hands-on-time to less than 1 min per sample.

The objective of this study was to compare and study the performance of the new GenMark ePlex assay against the in house real-time PCR, a lab developed test (LDT).

Material and methods: 81 nasopharyngeal swabs samples (NPS) in UTM were previously tested by an in-house Real Time PCR. Samples selected contained the following respiratory pathogens: respiratory syncytial virus, influenza A, influenza B, rhinovirus, enterovirus, bocavirus, coronaviruses, metapneumovirus, parainfluenza viruses, *Bordetella pertussis* and *Mycoplasma pneumoniae* 32.1% samples were co-infected, even with 4 different organisms. Samples selected were less than 4 months old with only one freeze/thaw cycle. Ct values ranging from 17.11 to 40.39 mean 24.74.

200 μl of each NPS sample was added to the ePlex Sample Buffer device, transferred to the RP cartridge and then inserted on the ePlex device.

Agreement between the original LDT results and the results obtained with the ePlex assay was assessed as detected or not detected.

Additionally 5 successive 1:10 dilutions were performed for 7 different specimens: RSV, influenza A H1N1, influenza B, rhinovirus, bocavirus, *Mycoplasma pneumoniae* and *Bordetella pertussis*. Dilutions were tested on both assays to identify and compare the lower limit of detection to the LDT.

Results: Total concordance was observed in 91.73% of cases. Only 10 discrepancies were identified. 7 organisms were detected by the ePlex assay and missed by the LDT and 3 organisms were positive detected by the LDT and negative by the ePlex. Discrepancies were repeated in both assays showing same results.

Total concordance was observed in 80% of dilutions. 1 dilution 10^{-4} RSV sample was detected by the ePlex and resulted negative for the LDT. As well 6 samples (10^{-3}–10^{-5} dilutions) were positive for the LDT and negative for the ePlex assay. *B. pertussis* did not detect the 2 lower dilutions as a different target gene was in use in ePlex assay.

Conclusion: The preliminary evaluation on a small sample set show a very good agreement across a range of pathogens with the GenMark compares in house real-time PCR. The assay was also found to be very simple and easy to perform and would be suitable for a hospital ward or outpatient environment.

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Single genetic clades of EV-D68 strains in 2010, 2013, and 2015 in Osaka City, Japan

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Background: Detection of Enterovirus D68 (EV-D68), a cause of acute respiratory tract infection (ARTI), was rarely reported before the early 2000s. Molecular analyses have demonstrated that recently detected EV-D68 strains are of three major genetic clades. We previously reported the emergence of EV-D68 in children with ARTI in Osaka City, Japan in 2010.

Objectives: This study surveyed EV-D68 among children with ARTI since its first endemic period in 2010 and conducted molecular analyses of the detected viral genome sequences.

Methods: During November 2010–December 2015, 2215 respiratory clinical specimens were obtained from children (<10 years old) with ARTI. Specimens from patients diagnosed with influenza were excluded. Real-time RT-PCR was used to detect enteroviruses. Viral protein 4 (VP4) or VP1 genes were sequenced to identify EV-

D68. To analyze and compare the EV-D68 endemics in 2010, 2013, and 2015 in Osaka City, complete or nearly complete viral genome sequences were determined. Then phylogenetic analyses were conducted.

Results: Enteroviruses were detected in 119 (5.4%) samples. Specimens positive for EV-D68 were 18 (0.8%, 18/2215): 4 in 2013 (2.8%, 4/142), 1 in 2014 (0.4%, 1/232), and 13 in 2015 (1.9%, 13/675). All EV-D68 strains, except for one strain in 2014, were detected during summer–autumn. About 94% of EV-D68-positive patients had lower respiratory tract infections such as bronchitis, asthmatic bronchitis, and pneumonia. Phylogenetic analyses using available VP1 sequences revealed that EV-D68 strains detected in Osaka City in 2010, 2013, and 2015 belong to distinct clusters: Clades C, A, and B, respectively. Genetic clades were named according to an earlier report [Tokarz et al., J Gen Virol., 2012]. Complete or nearly complete genome sequence determination revealed that Osaka strains of Clades A, B, and C commonly have deletion corresponding to nucleic acid positions 681–704 in the 5′ untranslated region (UTR) of the prototype Fermon strain. Both Clades B and C have additional deleted regions in the 716–727 nt position.

Conclusions: Re-emergence of EV-D68 was observed mainly in 2013 and 2015 since its first endemic period in 2010 in Osaka City. Although some specific deleted nucleotide sequence regions were observed in 5′ UTR among three genetic clades compared with the prototype strain, their functional differences and importance remain unclear. Genetic differences between EV-D68 strains in 2010, 2013, and 2015 might contribute to the resurgence of EV-D68 every few years in the limited geographic region.

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Influenza surveillance during 2015/2016 season in Portugal

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Background: The National Influenza Surveillance Program ensures influenza epidemiological surveillance, integrating clinical and laboratory data. The clinical component describes the transmissibility over time, the virological component detects and characterizes influenza virus in circulation. Data is generated through 2 sentinel surveillance structures, the General Practitioner's Sentinel Network (since 1990) and the Network of Emergency Units (since 1999). Here we provide the evaluation of 2015/2016 influenza season in Portugal.

Methods: Influenza-Like illness (ILI) cases were reported to the National Influenza Reference Laboratory and to the Epidemiology Department of the National Institute of Health, in the context of the National Influenza Surveillance Program, from week 39/2015 through week 20/2016. The intensity and duration of the epidemic period were described based on the weekly ILI incidence rates. Nasopharyngeal swabs were collected for influenza and other respiratory viruses (RV: respiratory syncytial virus, adenovirus, rhinovirus, metapneumovirus, coronavirus, parainfluenza virus) for diagnosis and characterization. The detection of influenza and RV was performed by multiplex real-time RT-PCR. Influenza virus isolation, antigenic analysis (hemagglutination inhibition assay) and genetic characterization (HA1 gene segment) were performed.

Results: During 2015/2016 season the influenza activity was low and the epidemic period occurred between week 1/2016 and 9/2016 with a maximum of 59.4 ILI cases per 10^5 inhabitants in week 3/2016. No impact on mortality from all causes was observed in any age group. 1097 nasopharyngeal swabs were tested for influenza and other respiratory viruses, influenza was detected in 444 (41%) and others respiratory viruses were found in 265 (24%). The influenza A(H1)pdm09 was predominant (91% of flu confirmed cases). Influenza B/Victoria was identified sporadically (7% of flu cases) in late season. A(H1)pdm09 were predominantly detected in adults aged 15–64. Other respiratory viruses were detected in influenza negative cases, being rhinovirus (101; 38%) and coronavirus (78; 29%) found in higher frequencies. The antigenic and genetic analysis of circulating influenza A(H1)pdm09 showed similarity with vaccine strain. The majority of influenza B virus belonged to Victoria lineage and clade 1A, dissimilar from 2015/16 vaccine strain. Although few A(H3) viruses in circulation, almost all were similar to strain recommended for next season, 2016/2017, influenza vaccine. None of the 420 A(H1)pdm09 viruses analysed showed the H275Y substitution, correlated to high reduced susceptibility to oseltamivir.

Conclusions: Influenza activity during 2015/2016 flu season was low, that can be linked with a higher influenza activity in last season and exceptional climatic conditions during the winter (higher temperature than usual). A(H1pdm)09 viruses were dominant, although in co-circulation with influenza B/Victoria. Situation that contrasts with European influenza picture, that showed a late peak of influenza B/Victoria. Influenza A(H1)pdm09 detections reached the highest percentage in adults (15–64 years old). Most influenza detected viruses were similar to the 2015/2016 vaccine strains, although circulating influenza B/Victoria were from a different lineage comparing with vaccine strain. Observed mortality from all causes was within expected values during study period.

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Molecular characterization of respiratory syncytial virus during 2015–2016 season in Portugal

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Background: Respiratory syncytial virus (RSV) is one of the most frequent and important respiratory viral agent that causes respiratory infection complications in younger children and elderly. RSV has an autumn/winter seasonality. Genetic diversity in both of RSV A and B subtypes increased in last years with the spread of new genotypes. This study aims to describe the genetic variability of RSV during 2015/2016 season in Portugal and correlate the circulating genotypes with detected ones in previous seasons. Will also be evaluated the association between genotype, clinical diagnosis and age.

Methods: During 2015/2016 winter season, between November/2015 and February/2016, 45 RSV were genetically characterized. RSV positive respiratory samples were collected in two settings: children under 5 years old diagnosed by hospital laboratories from the Portuguese Laboratory Network for the Diagnosis of Influenza Infection, and all age Influenza-like illness (ILI) patients reported by primary care health services and diagnosed by the National Influenza Reference Laboratory. All samples were irreversibly anonymized. Demographic and clinical data were collected. RSV detection was performed by real-time PCR and other biomolecular methods. RSV genotype was assigned by the nucleotide sequence of the hypervariable C-terminal region of the G protein gene and the phylogenetic analysis was performed in MEGA 6.0.

Results: From 45 RSV genetically characterized, 31 (69%) were reported by hospitals, patients age ranged from newborn to 4 years old. From these, 25 (81%; 25/31) patients were hospitalized, being the bronchiolitis the most frequent diagnosis. While 14 (31%) RSV cases came from primary care health services, patients age ranged from 3 to 83 and all had a clinical diagnosis of ILI. Were included patients from both genders in equal proportions.

RSV A and B co-circulated during 2015/2016 season. Were genetically characterized 21 (47%) RSV A and 24 (53%) RSV B. 90% (19/21) of RSV A clustered in ON1 genotype, the others 2 clustered with NA1 genotype. All RSV B present a BA-like genotype, 70% (17/24) were similar to BA9 and 30% (7/24) clustered with BA10 genotype.

Conclusions: During 2015/2016 season was observed a co-circulation of RSVA and RSVB. In present study ON1genotype was predominant in circulation among RSVA, this was also detected as the major RSVA genotype at the global level. Only two RSVA belonged to NA1 genotype. In Portugal, NA1 was in circulation during 2010–2012 period. Undetected since 2012, it seems to reappear during 2015/2016 season. All RSVB characterized belonged to BA genotypes, the majority clustered within BA9 genotype. BA10 genotype was also identified in circulation at low frequency. BA9 and BA10 were being found in co-circulation since 2011/2012. No association was found between age, clinical diagnosis and RSVA and B genotypes. RSV has an important impact in children in high-risk groups highlighting the need of a continuous RSV surveillance each winter.

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Circulating enterovirus genotypes in Norway, 2014–2015: A reason for concern?

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Background: Enteroviruses (EV) are small single-stranded RNA viruses belonging to the *Picornaviridae* family, and are responsible for a wide variety of human infections ranging from mild respiratory illnesses to severe neurological diseases with central nervous system involvement. In Norway, typing of enterovirus is performed both at the Virology Department of the Norwegian Institute of Public Health (NIPH) which is the National/WHO Reference Laboratory for Polio/Enterovirus receiving samples from all regions of the country and at St. Olav's Hospital. Following an alarming detection of EV-D68 infections in young children presenting with acute flaccid paralysis (AFP) in Norway in 2014, the aim of this study was to monitor the circulating EV genotypes obtained from the available data collected from 2014 and 2015, in Norway.

Methods: We performed a retrospective descriptive study on EV cases with clinical information dating from 2014 to 2015 by analysing the distribution of isolates identified by serotyping using neutralisation with monovalent antisera at NIPH; or identified by Sanger sequencing at St. Olav's Hospital. Statistical analysis was performed using STATA software, with significance at $p < 0.05$.

Results: In general, the majority of EV positives was verified among male (ranging from 52–56%) children below 2 years of age. The analysis performed on NIPH data revealed that 23/132 (17.4%) and 36/101 (35.5%) of samples were EV positive in 2014 and 2015, respectively, of which 18/23 (78.3%) and 34/36 (94.4%) were typed. The overall typing analysis for both years showed a predominance of echo 18 and echo 30, with a threefold increase of Coxsackie B5 from 2014 to 2015. For St. Olav's 2015 data, we verified that, from a total of 1183 samples, 146 (12.3%) were EV positive, from which

Coxsackie A6 (20%), EV71 (4.8%) and echo 3 (3.4%) were the most commonly detected. Analysis of clinical data showed a significant association between hand-foot-mouth disease (HFMD) and genotypes EV71 (risk ratio, rr13.6; Confidence interval, CI 5.4–33.9) and Coxsackie A6 (rr29.4; CI 17.7–48.8). In 2014, Coxsackie A16 was found in stool samples from one AFP case and EV-D68 was found in respiratory specimens from two AFP cases.

Conclusion: This study shows the seasonal fluctuation in circulating EV genotypes from one year to the next, verified in prevalence switches mainly between Coxsackievirus and echovirus genotypes. In addition to the well-known association between HFMD and EV71, we detected an association between Coxsackie A6 and HFMD, which has of late also been observed in other countries. These findings highlight the importance of continued EV surveillance in order to establish the virulence of circulating and upcoming EV genotypes and appropriately guide specific Public Health recommendations.

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Different epidemiological characteristics of respiratory virus infections in children and adults

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Acute respiratory tract infections (RTI) are mostly caused by viruses and are a leading cause of morbidity and mortality especially in young children, the elderly, and in immunocompromised patients. We analyzed epidemiological characteristics of RTI in a university hospital setting in the winter seasons 2015 and 2016.

Diagnostics of respiratory viruses was performed prospectively with the multiplex PCR "FTD Respiratory Pathogens 21" (Fast-track Diagnostics). This kit allows detection of influenza A and B virus (Flu A and B), respiratory syncytial virus (RSV), metapneumovirus (MPV), adenovirus (AdV), coronaviruses (CoV) 229E/OC43/NL63/HKU1, parainfluenzaviruses (PIV) 1-4, rhinovirus, enterovirus, parechovirus and bocavirus (BoV). Results of respiratory virus testing were analyzed of all samples received from the hospital of the University of Würzburg, Germany, during the period January 2015 to April 2016. For data analysis, the study period was subdivided into first season (January 2015 to June 2015) and second season (July 2015 to April 2016).

During the study period, 4136 respiratory samples from 2905 patients at the university hospital Würzburg were tested for the presence of respiratory viruses by multiplex PCR, 2948 in the first season and 1188 in the second season. The median age of the patients was 50.3 years (range 0.01–98.3). Of the 2905 patients 1139 (39.2%) were children. The male versus female ratio was 1.34:1. The overall positivity rate was 37.8% in the first season, 47.1% in the second season, and 40.4% during the whole study period. The positivity rate in children was significantly higher than in adults (61.4% versus 29.5%). Similarly, the rate of double virus detections was significantly higher in children than in adults (17.8% versus 6.5%). Detection of three or more viruses in one sample was only observed in children (4.6%). In both seasons the leading virus detected in respiratory samples was rhinovirus with 27.9% and 25.4%, respectively, of all positive samples. Rhinovirus was followed by Flu A (21.7%), RSV (15.8%), and PIV (12.9%) during the first season and by RSV (18.8%), CoV (17.4%), Flu A (14.7%), and MPV (12.7%) during the second season.

The virus distributions in both seasons were considerably different between children and adults.

In summary, comparison of results of respiratory virus diagnostics in children and adult populations shows substantial differences, which demonstrates the need and usefulness of multiplex PCR for broad spectrum detection of respiratory viruses.

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Abstract no: 313
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Presence of human bocavirus 1 and other viral co-infections in hospitalized children with lower respiratory tract infection in Latvia

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Background: Acute respiratory tract infection (ARTI), especially lower respiratory tract infection (LRTI), is the common cause of illness and hospitalization in children worldwide. However, in many cases the etiological agent of disease is unknown. The viruses primarily associated with respiratory tract infections in children are respiratory syncytial viruses, influenza viruses, parainfluenza viruses, adenoviruses, coronaviruses, rhinoviruses and enteroviruses. In recent years the role of several new respiratory viruses in respiratory tract diseases have been reported, including human metapneumovirus, four coronaviruses (SARS-CoV, HCoV-NL63, HCoV-HKU1, MERS-CoV) and human bocavirus 1 (HBoV1). The aim of this study was to determine the presence of HBoV1 and 18 other respiratory viruses in nasopharyngeal aspirates (NPAs) from hospitalized children with LRTI in Latvia.

Material and methods: Forty four children (28 male and 16 female) aged one to 50 months who were hospitalized in Children's Clinical University Hospital and fulfilled WHO LRTI criteria plus had fever ($T \geq 380\,^{\circ}C$) were enrolled in this study. In all cases the etiological agent of the disease was not revealed using standard routine clinical methods. NPAs from all patients were obtained on admission and DNA from NPAs was extracted using phenol-chloroform method. All 44 DNA samples were tested for HBoV1 and 18 other respiratory viruses (influenza viruses A, A-H1, A-H1pdm09, A-H3 and B, respiratory syncytial viruses A and B, adenovirus, enterovirus, parainfluenza viruses 1–4, metapneumovirus, rhinovirus, coronaviruses NL63, 229E and OC43) using multiplex real-time PCR method.

Results: Among 44 patients with LRTI, 29 (65.9%) were positive for HBoV1 which was the most frequently detected virus in patients. However, only HBoV1 genomic sequence without any analysed coinfection was detected in two out of 29 (6.9%) patients. Respiratory syncytial virus A was found in 23 out of 44 (52.3%) DNA samples and it was the most common co-infection. Other respiratory viruses detected were: adenovirus in 14 (31.8%), rhinovirus in 9 (20.5%), respiratory syncytial virus B in 7 (15.9%), metapneumovirus in 3 (6.8%), parainfluenza virus 3 in 2 (4.5%), coronavirus 229E in 2 (4.5%), (enterovirus in 1, influenza A virus in 1, influenza B virus in 1), coronavirus OC43 in 1 and coronavirus NL63 in 1 patient with LRTI. In 13 cases presence of more than two respiratory pathogens were found and in two cases, none of the tested respiratory viruses were detected.

Conclusion: HBoV1 DNA is frequently found in NPAs from children with LRTI in Latvia. Although very often HBoV1 infection is accompanied by co-infections with other respiratory viruses, however there are LRTI cases when HBoV1 is the only pathogen detected, indicating its possible role in etiology of the disease.

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Abstract no: 316
Presentation at ESCV 2016: Poster 208

Molecular epidemiology of circulating human coronaviruses in children at a tertiary hospital in Catalonia (Spain) from 2014 to 2016

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Background: Human Coronaviruses (HCoVs) are single-stranded, positive-sense RNA viruses. Four HCoVs species (229E, OC43, NL63 and HKU1) are currently associated with asymptomatic or mild upper-respiratory tract infections (URTI) in general population, but severe acute respiratory infection (SARI) may occur in patients with high risk of infection, such as immunocompromised patients. The main aim of this study was to describe the seasonality and genetic diversity of HCoVs, and the clinical features related to HCoVs infection, in paediatric patients attended in our hospital from 2014 to 2016.

Methods: From October 2014 (week 40) to May 2016 (week 20) respiratory specimens were collected from paediatric patients who were attended at the emergency care unit, outpatient departments or admitted to Hospital Universitari Vall d'Hebron (Barcelona, Spain) for diagnosis of respiratory viruses by Anyplex II RV16 Detection Kit (Seegene, Korea), that is only able to detect HCoV-229E, HCoV-OC43 and HCoV-NL63, in addition to other respiratory viruses. Partial RNA-dependent RNA polymerase gene (RdRp) was sequenced from laboratory – confirmed HCoVs specimens for subsequent phylogenetic analysis in order to confirm the routine diagnostic PCR results. In addition, partial coding sequence of the spike (S) glycoprotein was sequenced to identify the different HCoV genotypes. Clinical and epidemiological features of HCoV infected cases were retrospectively reviewed from medical records.

Results: A total of 6661 specimens from 3900 patients were received at our laboratory, of which 117 (2%) from 96 patients were positive for HCoVs (11 for HCoV-229E, 12%; 33 for HCoV-NL63, 34% and 52 for HCoV-OC43, 54%). But, phylogenetic analysis of 61 partial RdRp sequences revealed that viruses were belonging to the four species (6 HCoV-229E, 9%; 15 HCoV-NL63, 25%; 22 HCoV-OC43, 36%; and 18 HCoV-HKU1, 30%). HCoVs circulated throughout the year, but highest number of detections were shown in autumn months. Based on phylogenetic analysis of 69 S sequences: HCoV-NL63 (32) fell into two clusters (16 A, 50%; 16 B, 50%); HCoV-OC43 sequences (19) in two clusters (5 B, 26%; 14 C, 74%); and HCoV-HKU1 (18) mainly in other two (16 A, 90%; 1 B, 5%), but one (5%) out of known genetic subgroups.

HCoV was more often found in respiratory samples of children with URTI: 58% had URTI, of which 21% were associated with lower respiratory tract infection (LRTI); 20.5% of patients had LRTI without URTI; and, 21.5% were asymptomatic. HCoV-HKU1 (20%) and HCoV-OC43 (29%) URTIs were less associated with LRTI than HCoV-229E (50%) and HCoV-NL63 (40%). Most of children admitted with HCoV LRTI required supplemental oxygen (11 out of 17 hospitalised patients), but only 2 required it for more than 4 days. HCoV-229E was related with more oxygen requirements, and HCoV-OC43 with longer hospitalization stays. Only one case was admitted to Paediatric Intensive Care Unit. No fatal cases due to HCoV infection were reported.

Conclusions: Simultaneous circulation of the several HCoVs species was shown from 2014 to 2016. Phylogenetic analysis revealed the circulation of viruses belonging to different genetic subgroups. Despite seasonal infection by these four HCoV species is usually related to mild–respiratory disease, little differences in the clinical features per specie were shown. Virological surveillance must be done to detect changes on the virological and clinical features related to circulating viruses.

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Abstract no: 319
Presentation at ESCV 2016: Poster 209

No substantial circulation of enterovirus D68 in patients with severe respiratory disease in South-eastern Spain (Valencian Community) during the 2015–2016 influenza season

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Background: Enterovirus-D68 (EV-D68) was associated with severe respiratory disease in North America and other geographical regions during the fall of 2014.

Methods: We compared the detection rates of EV-D68 in the 2014-2015 influenza season with that of the 2015-2016 season in samples collected in a prospective surveillance scheme for all hospitalizations due to respiratory disease in our region (Valencian Community, South-eastern Spain). Combined nasopharyngeal and nasal (children <14 yr. old) or nasopharyngeal and pharyngeal swabs are analyzed in a single laboratory at FISABIO-Public Health for 16 respiratory viruses by multiplex real-time RT-PCR, including rhinovirus/enterovirus as a single target. All samples positive for rhinovirus/enterovirus were retested with a rhinovirus/enterovirus discriminative real-time RT-PCR, and those enterovirus positive for EV-D68 specific detection as a single target.

Results: In the 2014–2015 season, between November 15th and March 31st, 372 of 4472 (8.32%) samples were rhino/enterovirus positive, of which 66 (17.75%) were identified as enterovirus, and 15 (4.03%) confirmed as EV-D68. In the 2015–2016 season, between November 15th and April 30th, 201 of 2700 (7.45%) samples were rhino/enterovirus positive, of which 42 (20.82%) were identified as enterovirus, and only one (0.50%) confirmed as EV-D68.

Conclusion: Based on testing results, and in contrast with the 2014–2015 influenza season, the circulation of EV-D68 in Southeastern Spain (Valencian Community) in cases of severe respiratory disease during the 2015–2016 season was almost absent. Further studies are needed to determine if these low detection rates have also decreased in other geographical regions.

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Abstract no: 321
Presentation at ESCV 2016: Poster 210

Genetic variability of human metapneumovirus A strain circulating in Catalonia during the 2014–2015 and 2015–2016 seasons: A 180-nucleotide G gene duplication reported

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Background: Human Metapneumovirus (HMPV) causes respiratory tract infections (RTI) in children. HMPV is an enveloped single-stranded negative-sense RNA virus. It usually shows a seasonality pattern, mainly in spring and winter months. Fusion (F) and glycosylate (G) proteins, the two major envelope proteins of the virus, evolve by selective immune pressure. HMPV is divided into two genotypes (HMPV-A and -B), into 4 subgenotypes (A1, A2, B1 and B2), and also into two lineages (A2a and A2b). The aim of this study was to describe the molecular epidemiology and diversity of HMPV, and the clinical features related to infection, in paediatric population attended at a tertiary university hospital in Barcelona from 2014 to 2016.

Material and methods: Respiratory specimens from paediatric patients with suspicion of RTI attended at Emergency Care Unit or outpatient departments, or admitted to Hospital Universitari Vall d'Hebron (Barcelona, Spain) were collected from October 2014 (week 40) to May 2016 (week 20) for virological diagnosis. All samples were laboratory-confirmed for HMPV by immunofluorescence or by real-time multiplex RT-PCR assays. Both complete G and partial F coding sequences from HMPV viruses were sequenced to perform phylogenetic analyses and molecular characterisations with MEGA v5.2. Clinical and epidemiological features of HMPV infected cases were retrospectively reviewed from medical records.

Results: A total of 6658 specimens from 4488 paediatric patients were collected, of which 128 (2%) samples from 121 (3%) patients were laboratory-confirmed for HMPV. Based on phylogenetic analysis of G or F sequences, 59 (49%) viruses belonged to HMPV-A and 54 (45%) to HMPV-B genotypes; the remaining 8 viruses (6%) could not be sequenced. Weekly distribution of HMPV detections showed a higher circulation from February to April. Although both HMPV genotypes simultaneously co-circulated, HMPV-B was predominant in the 2014–2015 season while HMPV-A was in the 2015–2016 season. Regarding the HMPV-A phylogenetic analysis, 52 (88%) belonged to A2b lineage and 7 (12%) to A2a. In addition, molecular characterisation of A2b sequences revealed that 9 sequences (2014–2015, 2; 2015–2016, 7) had a 180-nucleotide (60 amino acids) duplication in the G protein.

Overall, HMPV caused lower respiratory tract infections (LRTI) in 74% of children, more frequently for genotypes A2b (87%) and B2 (81%). 85% of patients required hospitalisation (median: 8.3 days). Children with B1 LRTIs, compared with other genotypes, had longer hospital stays (median: 8.8 days) and longer supplementary oxygen requirements (median: 4.6 days), but one required mechanical ventilation (MV). 6 children with A2b LRTI and 3 with B2 LRTI required MV. No fatal cases due to HPMV infection were reported. Most children with A2b with 180-nt duplication had LRTI (67%), who all required hospitalization (3 with supplementary oxygen, and one with MV).

Conclusions: Recent and valuable data on regards on seasonality, genetic diversity and clinical features of circulating HMPV in Catalonia (Spain) is reported. A 180-nucleotide duplication within HMPV G protein is first described here to our knowledge. The fact that the number of viruses with this 180-nt duplication increased during the last season suggests that it may become predominant in the future. In addition, further studies are needed to know if this variant causes different disease severity.

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Respiratory viruses in patients with acute respiratory infections in the pediatric and adults intensive care units

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Background: Severe Acute Respiratory Infections (ARI) that requires intensive care unit (ICU) admission is associated with high morbidity and mortality. Patients with severe ARI admitted to the ICU, are usually monitored a standard approach consisting of bacterial culture and testing. The importance of viruses in severe ARI has been apparent with new viral detection techniques in recent years. The aim of this study was to investigate the prevalance of respiratory viruses in patients with severe ARI who required admission to a medical ICU during the 2015–2016 winter season.

Methods: Between September 2015 and April 2016, nasopharyngeal swab samples were collected from 70 children (36 female and 34 male) and 18 adults (6 female and 12 male) with ARI in Pediatric and Adult Intensive Care Units of Akdeniz University Hospital. Samples were investigated by multiplex PCR (Verigene Respiratory Pathogens *Flex* Test; RP *Flex*; Nanosphere, Northbrook) for Adenovirus, Metapneumovirus, Influenza (INF) A, (subtype AH1 and AH3), INF B, Parainfluenza 1,2,3 and 4, Rhinovirus, RSV A and B, *Bordetella sp.*

Results: The median age was 1 year (range: 1 day–84 year) of the patients. Positive samples were found between October 2015 and March 2016 and most frequently positivity rates were detected in January (50%), February (73.7%) and March (50%). The chronic diseases were common in the positive patients (51.3%) such as cardiac diseases, chronic pulmonary disease, diabetes mellitus. About 47.7% of patients were positive for at least one viral pathogen. Single viral pathogen occurred in 73.8%, viral coinfections were found in 26.2% of positive patients. Influenza viruses were the most common

Table 1
Demographic and clinical characteristics of 42 ICU patients with ARI and positive for viral pathogen.

Characteristics	Children ($n=28$) (%)	Adult ($n=14$) (%)	Total ($n=42$) (%)
Male	13 (46.4)	10 (71.4)	23 (54.8)
Female	15 (53.6)	4 (28.6)	19 (45.2)
Age, median (range)	49.5 day (4 day–15.4 year)	52.7 year (23–84 year)	1.33 year (4 day–84 year)
Pneumonia	7 (25.0)	5 (35.7)	12 (28.6)
Respiratory failure	12 (42.8)	8 (57.1)	20 (47.6)
Bronchiolitis	5 (17.8)	0[a]	5 (11.9)
Other	4 (14.3)	1[a]	5 (11.9)
Oxygen support	15 (53.6)	12 (85.7)	27 (64.3)
Mechanical ventilation	4 (14.3)	9 (64.3)	13 (30.9)
Mortality rates	2 (7.1)	4 (28.6)	6 (35.7)
INF viruses	9 (32.1)	12 (85.7)	21 (50.0)
INF A/H1	3 (10.7)	8 (57.1)	11 (26.2)
INF A/H3	2 (7.1)	1[a]	4 (9.5)
INF A	1[a]	1[a]	2 (4.8)
INF B	3 (10.7)	2 (14.3)	7 (16.6)
Rhinovirus	9 (32.1)	5 (35.7)	14 (33.3)
RSV	9 (32.1)	4 (28.6)	13 (30.9)
Parainfluenza virus 1, 3	3 (10.7)	1[a]	4 (9.5)
Adenovirus	2 (7.1)	1[a]	3 (7.1)
Metapneumovirus	1[a]	0	1[a]
Viral coinfections	5 (17.8)	6 (42.8)	11 (26.2)

[a] Not calculated.

viral pathogens (50.0%), especially among adults (85.7%). In children, influenza viruses, RSV and rhinovirus (32.1%) were detected frequently (Table 1).

Conclusion: Respiratory viruses are significant causes of severe ARI in adults and young children during the winter season. Early recognition of viral pathogens in ARI etiology is important in order to diagnosis and management of severe ARI in ICUs.

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Abstract no: 332
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Identification of mutations in surface glycoprotein genes of human respiratory syncytial virus in children treated with palivizumab

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Background: Palivizumab is a respiratory syncitial virus (RSV)-neutralizing monoclonal antibody clinically used for the prevention of severe RSV infections in high-risk infants, preterm infants and infants with hemodynamically significant heart disease or chronic lung disease. Palivizumab acts by blocking the fusion step of virus replication. Mutants resistant to palivizumab were isolated in vitro and also in children with RSV infection while receiving palivizumab. The mutations reported are situated on the fusion protein (amino acids 262–276), in the liaison site of the palivizumab. It seems that mutations out of the liaison site do not confer resistance to palivizumab. The gene coding for the attachement glycoprotein (G gene) was rarely sequenced. The aim of this study was to analyze the complete F and G genes sequences coding the surface glycoproteins of RSV isolates collected from patients receiving palivizumab.

Material and methods: RSV isolates were obtained from nasopharyngeal swabs of high-risk infants treated with palivizumab at the University Hospital of Caen between October 2011 and April 2016 and having presented a RSV-breakthrough during the treatment or in the six months after. RSV controls were obtained from infants who did not receive palivizumab. Viral ARN was extracted using Qiasymphony DSP Virus/Pathogen Mini kit®. The group typing of hRSV, A or B, was completed using real time RT-PCR. The amplification and sequencing of the complete F and G genes were performed using One-Step RT-PCR kit® (Qiagen, Hilden, Germany) and specific primers and protocols. The analysis and comparison of the obtained sequences with reference strains and control sequences were performed with BioEdit® software. Phylogenetic tree were constructed by the neighbor-joining method in MEGA 6.0® software.

Results: Among the 273 infants treated with palivizumab during the period of the study, 15 (8.4%) have presented a RSV infection during their treatment or in the six months after. Seven RSV/A and 8 RSV/B were identified by real-time PCR. The amplification and sequencing of the F and G genes were successfully undertaken.

For the RSV/A analysis, phylogenetic trees were constructed using 6 RSV/A detected, one control RSV/A and 42 reference sequences. The hRSV/A isolated in 2014 or after were identified in the ON1 cluster. When they were detected in 2011 they clustered with the GA2 genotype. None RSV/A was detected between 2011 and 2014.

The analysis of complete F genes alignments of hRSV/A shows several mutations out of the liaison site of palivizumab. We found one mutation in the liaison site, the N276S mutation. This was previously described as a mutation conferring partial resistance to palivizumab *in vitro* and *in vivo*. This mutation was also identified in the viruses collected from the control population.

Conclusion: This study allowed us to characterize mutations of RSV in case of palivizumab treatment failure.

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The use of in vitro human airway epithelia for the development of novel antivirals

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The human airway epithelium occupy a central position in the pathogenesis of respiratory viruses. As the first line of defense against microorganisms, epithelia cells reacts through mucus secretion, mucociliary clearance, activation and release of chemokines, cytokines, lipids, growth factors, proteases, etc.

Viral respiratory infections are the most frequent etiologies of acute illnesses worldwide and cause mild to severe diseases such as common cold, bronchiolitis and pneumonia. A comparative study was carried out on the infectivity and replication of the most frequent human respiratory viruses using standardized *in vitro*

reconstituted human airway epithelia (MucilAir™). Differentiated tissues were infected in parallel with clinically relevant strains of rhinovirus (A16, A49, A55, B48, C8, C15), respiratory enterovirus (EV68), influenza virus (H3N2) and corona virus (OC43). For each virus, replication kinetics, cell tropism, impact of the virus on tissue integrity and cilia function were assessed.

Development and use of anti-viral drugs are one of the priorities for major pharmaceutical companies. As proof-of-concept for drug screening, the efficacy of Rupintruvir and Oseltamivir were tested in MucilAir™. Rupintruvir efficiently inhibited the replication of HRV-A16 and HRV-C15 in a dose and time dependent manner (up to 99% inhibition). Interestingly, (i) Oseltamivir reduced the replication of H1N1 and H3N2 and restored the impaired barrier function monitored by Trans-Epithelial Electrical Resistance and (ii) Rupintivir restored the mucociliary clearance impaired by EV68 (7 μm/s for the Mock up to 40 μm/s for the Rupintrivir treatment at 50 nM at 96 h post innoculation).

These results demonstrated that MucilAir™ is a robust, reliable and relevant tool for antiviral drug development.

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Abstract no: 346
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Study on immunological characteristics of monoclonal antibodies produced against the Kazakhstan isolates of influenza A(H1N1) virus

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The main economic and social damage resulting from infectious diseases throughout the world is caused by acute respiratory viral infections and influenza. In recent years, the epidemic process is characterized by co-circulation of influenza virus subtypes A(H1N1), A(H3N2) and type B. In determining the etiology of viral infection serological analysis is one of the fundamental components. Monoclonal antibodies (MAbs) permit to dramatically increase the specificity and sensitivity of diagnostic techniques for the detection of viral antigens. Immunological characteristics of MAbs produced against the Kazakhstan isolates of influenza A(H1N1) virus were studied with immunofluorescence, HAI and microneutralization assays. Immunofluorescence testing revealed that MAbs are specific against homologous and related antigens, and identified them in the form of distinct granular fluorescence before the conjugate dilutions of 1:80–1:160. It was found that MAbs in HAI assay revealed a wide range of responses and in high titres (1:160–1:10240) inhibited the hemagglutinating activity of the homologous and related reference and Kazakhstan influenza viruses and did not react with the heterologous A(H3N2) and type B viruses. In microneutralization assay MAbs neutralized influenza A(H1N1) viruses and did not react with influenza viruses A(H3N2) and type B. Thereby, the similar spectra of MAb reactivity against A/H1N1 viruses indicate the presence of antigenic determinants in the HA composition of all the investigated viruses, that allows to recommend the resulting MAbs for differentiation of A(H1N1) viruses from the seasonal A(H3N2) and type B strains.

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Abstract no: 38
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Genetic diversity and characteristics of porcine reproductive and respiratory syndrome virus in the area of Korea from 2013 to 2015

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is rapidly gaining importance as one of the most economically significant diseases in swine worldwide. PRRSV is an enveloped positive single-stranded RNA virus that can be divided into two different genotypes, the European genotype (type 1) and the North American genotype (type 2). The genome of PRRSV is approximately 15 kb in length and contains at least 20 open reading frames (ORFs). ORF5, encoding GP5, is one of the most variable regions of the PRRSV genome, and often used to examine genetic diversity and monitor evolution of PRRSV. In this study, the recent isolates in the field were evaluated for genetic variation based on ORF5 nucleotide and amino acid sequence.

Materials and methods: Lung and serum samples were collected from 541 pig farms in nationwide where clinical symptoms had been observed in 2013–2015. Total RNA was extracted from serum and lung using an RNeasy mini Kit (QIAGEN) according to the manufacturer's protocol. To obtain sequences of the complete ORF5, reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using the One Step RT-PCR Kit (QIAGEN) and PRRSV primer sets derived from sequences of the ORF4-6 of the LV and VR-2332 strain, respectively. Multiple sequence alignments and phylogenetic trees were carried out using CLC Main Workbench 7.0.3 and Mega 6 program. Bootstrap values were calculated on 1000 replicates of the alignments to assess the confidence limits of the branching.

Results: To more totally understand the genetic diversity and characteristics of PRRSV in the area of Korea, we analyzed the open reading frame (ORF) 5 sequences of 323 (type 1) and 269 (type 2) of PRRSV from 2013 to 2015. The results showed that both types 1 and 2 have been circulating in Korea pig farms and that the regional rate of infection was more prevalent in Gyeongsangnam-do province in Korea. Type 1 PRRSVs from Korea are clustered in subtype 1, subgroup A, B, and C. Type 2 PRRSVs are classified in lineage 1, 4, 5 and new Korea subgroup A, B. Recently, the genetics of type 2 PRRSVs in Korea have become unique regional characteristics in Gyeongsangnam-do. Recently, the genetics of PRRSVs in Asia have become more diverse. Although the genetics of type 2 PRRSV in Korea have unique regional characteristics in Gyeongsangnam-do, the genetics of PRRSV in Asia have become more diverse.

Conclusions: This study of PRRSV in different geographical areas should be performed regularly to monitor field isolates. This would provide annual genetic information for PRRS control and vaccine selection and/or renewal [1,2].

Reference

[1] E.J. Choi, et al., Genetic diversity of porcine reproductive and respiratory syndrome virus in Korea, J. Vet. Sci. 14 (2) (2013) 115–124.
[2] S.H. Kim, et al., A molecular analysis of European porcine reproductive and respiratory syndrome virus isolated in South Korea, Vet. Microbiol. 143 (2010) 394–400.

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Abstract no: 42
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Cluster of severe influenza infections in a smoke-related environment

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Background: Influenza season 2015–2016 was characterized as severe influenza season with an increased incidence of ICU hospitalizations and fatalities at a younger mean age. Influenza A(H1N1)pdm09 predominated in Greece and the rest of Europe.

Methods: Laboratory influenza surveillance is performed at the National Influenza Centre for N Greece. RNA from pharyngeal and/or bronchial aspirates was extracted using the Qiamp RNA mini kit according to the manufacturer's instructions. Real-time RT-PCR was used for the detection and typing of influenza viruses, according to the CDC guidelines. RT-PCR was performed for the amplification of the viral haemaglutinin (HA), followed by Sanger Sequencing, as previously described by Melidou et al., 2015.

Conclusion: 104 patients admitted in ICU had a mean age of 55.9 years, while a total of 41 influenza related fatalities were reported at a mean age of 55.9 years. Interestingly, 30% of them reported no other underlying medical conditions. A cluster of influenza A(H1N1)pdm09 infections was observed during week 5/2016 in the Fire Department of N. Greece. One of the patients, aged 43, who was an otherwise healthy individual, suffered from ARDS and pneumonia and was eventually deceased, while a colleague of his, aged 44 and an otherwise healthy individual as well, was treated for ARDS in the ICU for one month. He had endotracheal intubation, timely administered oseltamivir and eventually recovered. Genetic analysis of the isolated influenza viruses revealed that the HA gene of the viruses belonged to the 6B.1 genetic group, and possessed no variations in antigenic or potential N-linked glycosylation sites, to which increased pathogenicity could be attributed. HA viruses also did not possess the D222G variation, previously associated with increased pathogenicity of influenza A(H1N1)pdm09 viruses. While genetic analysis of the whole viral genomes is pending, the importance of the work environment cannot be overlooked. Individuals with smoke-related activities/employment, that might affect their respiratory health, should be included in the high risk groups, strongly urged to vaccinate annually against influenza viruses and to timely use oseltamivir in the case of a suspected respiratory tract infection. The importance of employment reporting during national surveillance of influenza is highlighted.

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Prevalence of influenza virus types A and B in patients with acute respiratory infection: 2002–2015

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Objectives: In this study, we aimed to investigate the prevalence of influenza viruses in patients with the upper and lower respiratory tract infections of outpatient or inpatient monitoring who admitted to Ege University Medical Faculty Hospital.

Methods: Respiratory tract specimens were collected from 6665 patients [2911 (43.7%) outpatients and 3754 (56.3%) inpatients] with upper and lower respiratory tract infections between January 2002 and September 2015. The age range of patients [4983 (74.8%) pediatric and 1682 (25.2%) adult patients] is between five days to 94 years (median: 4 years). All specimens were tested by two or three assays [Direct fluorescent antibody (DFA), shell vial cell culture (SVCC), and multiplex PCR (mPCR)]. All specimens were tested by both DFA by using fluorescein isothiocynate labelled polyclonal antibody pool (Respiratory Screen Kit, Light Diagnostics, Chemicon International, USA) with cytospin and SVCC from 2002 to 2015. Isolation of influenza virus type A and B (INF-A, INF-B) were done by shell vial assay using MDCK cell line. Coverslips were stained with a fluorescein isothiocynate labelled monoclonal antibody specific for INF-A and INF-B (Light Diagnostics, Chemicon International, USA) according to the manufacturer's protocol. Three different mPCR kits (Respiratuvar RealAccurateTM, PathoFinder, Netherlands, Seeplex RV15 ACE Detection, and Annyplex II RV16 Detection, Seegene, South Korea) were used between 2007 and 2015.

Results: Of the 6665 specimens tested, 706 (10.6%) were found positive for INF-A and INF-B. In the 706 influenza virus positive specimens, 618 (87.5%) were INF-A and 88 (12.5%) were INF-B. In the group of 2911 outpatients, 404 (13.9%, INF-A 12.9%, INF-B %1.3) of them were positive, in the group of 3754 inpatients, 302 (8.0%, INF-A %6.7, INF-B %1.3) of them were positive for INF-A and INF-B. 476 (9.6%) patients were positive in the pediatric group and 230 (13.7%) patients were positive in the adult group for INF-A and INF-B. Influenza virus activity was started in October and it continued until the end of April in our region, it was observed that the peak was November–December–January period.

Conclusion: Influenza viruses were identified approximately 11% of patients with acute respiratory tract infection. Influenza viruses were detected more frequently in adult patients than pediatric patients. When the activity of influenza A and B virus compared; INF-A was found positive at higher rates. INF-A was isolated from more outpatients than inpatients. Influenza virus activity was observed most frequently in February in our region.

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Abstract no: 62
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Surveillance of a severe A(H1N1)pdm09 dominated influenza season in N. Greece, 2015–2016

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Objects of the research: The objective was the epidemiological and virological analysis of a severe influenza A(H1N1)pdm09-dominated season, 2015–2016 in N. Greece.

Materials and methods: 686 pharyngeal swabs/washes from patients with influenza-like-illness were tested up to week 17. Influenza viruses were typed and their haemaglutinin was sequenced. (CDC and WHO protocols). All of the samples were non-sentinel, mostly originating from outpatient and inpatient hospital clinics.

Results: 246 samples were positive for influenza. A and B viruses were detected in 220 and 24 samples respectively. B viruses appeared during the first and the last weeks of the season. Out of the A viruses, 206 were H1N1pdm09 and 8 were H3N2. Molecular analysis of B viruses revealed that B-Victoria lineage viruses dominated this season. A(H1N1)pdm09 viruses were A/California/7/2009(H1N1)pdm09-like, but with accumulating variations at antigenic and other HA sites, that designated them into two distinct phylogenetic clades, 6B.1 and 6B.2.

Samples ranged between 0 and 86 years of age, with an average 40.6 years. Sixty-seven ICU patients had an average age of 54.5 years and in all of them A(H1N1)pdm09 was detected and most suffered from underlying medical conditions, were obese or pregnant. Most common complications were ARDS and pneumonia. In total, 39 fatalities have been reported in northern Greece. All of them were attributed to A(H1N1)pdm09. Interestingly, 6 of the decedents did not suffer from any underlying medical condition, 10 of those were obese (26.%) and 25 were suffering from cardiological problems (64%). Complications and underlying medical conditions are mentioned in detail in Table 1.

Conclusions: Compared to the findings from previous studies from Greece, it seems that it was a severe A(H1N1)pdm09-dominated influenza season. This subtype seems to cause more severe influenza illness, in a younger age group, more often causing hospitalization to otherwise healthy individuals. Circulating strains are increasingly more divergent. Our findings confirmed the genetic instability of influenza type A(H1N1)pdm09 viruses and highlighted the importance of continuous surveillance for the effective management of viral epidemics. Variation observed in Greek and also in European B viruses prompted WHO to change the B vaccine component to B/Victoria.

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Enhancement of respiratory virus isolation from specimens using centrifugation and interferon inhibitors

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Virus isolation from clinical specimens is inevitable to identify the etiological agent and to investigate the epidemiological analysis. In addition, the viruses isolated are used as important sources in the fields from the basic research to the bioindustry, including therapeutics, and vaccines. However, the respiratory viruses in clinical specimens are not easily isolated in cell cultures. Thus, methods to speed or enhance virus isolation are urgently required. Many previous reports have proved that centrifugation during virus inoculation to cells increased virus yields and speeded the virus detection time. Recently, interferon inhibitors treatment has also been used to enhance virus infection by blocking the expression of interferons, modulators inhibiting virus replication in cells. We used interferon inhibitors with centrifuged cultures for the detection of metapneumovirus, human respiratory syncytial virus, and Middle East respiratory syndrome coronavirus. Combination of centrifugation and interferon inhibitor treatment significantly increased the virus replication and viruses detected earlier than the routine method. We also test and compare the virus isolation rates between the centrifugation/interferon inhibitor treatment culture and the mock-treated culture.

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Abstract no: 74
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Molecular characterisation of human coronaviruses from patients with respiratory disease in Slovenia

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Background: Coronaviruses (CoVs) are the largest enveloped single-strand RNA viruses and belong to the *Coronaviridae* family in the *Nidovirales* order and are divided into four genera named *Alphacoronaviruses*, *Betacoronaviruses* (divided into the four clades A to D), *Deltacoronaviruses* and *Gammacoronaviruses*, based on the phylogenetic distance of highly conserved domains. Until now six human coronaviruses have been identified and HCoV-OC43 is the most common human coronavirus and has high genetically diversity. Five genotypes of HCoV-OC43 (A to E) have been identified and genotype D has been dominant from 2004 to 2012. Until now only 90 complete genome sequences of HCoV-OC43 were available in GenBank. In this study, we investigate of the presence of different genotypes among HCoVs strains and comparison their potential similarity.

Methods: Patients hospitalized with acute respiratory tract infections were included in the study. All nasopharyngeal swabs were sent to the laboratory of the Institute of Microbiology

and Immunology, Faculty of Medicine, University of Ljubljana, for the routine detection of respiratory viruses, including respiratory syncytial virus (RSV), human rhinoviruses (hRV), human metapneumovirus (hMPV), human coronaviruses (HCoVs), human bocavirus (HBoV), adenoviruses (AdV), parainfluenza virus (PIV) and influenza viruses A and B (Flu A-B) by real-time RT-PCR. HCoVs positive samples with high viral load (low Ct value) and those negative for all other respiratory viruses were include into further testing by amplifying a 440-bp-long fragment of the highly conserved polymerase gene.

Results: From December 2013 to February 2016, a total 16686 nasopharyngeal swabs from patients with acute respiratory tract infections were enrolled in the study. From these 976 (5.8%) were positive for HCoVs and 523 (58.6%) were negative for RSV, hRV, hMPV, HCoVs, HBoV, AdV, PIV, Flu A and FluB by real-time RT-PCR. From 523 HCoVs positive sample 129 were further tested for all HCoVs species, including 47 HCoV-HKU1, 44 HCoV-OC43, 24 HCoV-NL63, 11 HCoV-229E, 1 HCoV-HKU1/HCoV-229E and 1 HCoV-NL63/HCoV-229E. Only HCoVs positive samples (HCoV-HKU1 and HCoV-OC43) with high viral load (Ct-value less than 30) were include into further testing. To characterize the overall diversity of coronavirus sequences, 65 sequences have been included in phylogenetic analysis; 31 sequences of HCoV-OC43 and 34 sequences of HCoV-HKU1.

Conclusions: Among four circulating HCoVs, HCoV-HKU1 and HCoV-OC43 seem to show the highest prevalence and incidence in hospitalized patients. The phylogenetic analysis shows that Slovenian human coronavirus strains from this study belong to the four clusters, two grouping HCoV-OC43 and two HCoV-HKU1. The present study draws genetically diversity of human coronaviruses in Slovenian hospitalized patients.

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Rapid diagnosis of respiratory viral infections in primary health care

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Background: Respiratory tract infections (RTI) are the most common acute problems in primary health care. RTIs are mainly of viral origin. The epidemiology of respiratory viruses in primary health care settings is scarcely reported, as diagnostic tests for RTIs are sporadically used by general practitioners (GP). Rapid, sensitive and specific identification of viral RTIs might assist diagnostic interpretation and potentially prevent inappropriate use of antibiotics.

Aim: To increase our insight in the epidemiology of viral RTIs in primary health care; to evaluate the feasibility and diagnostic accuracy of a new rapid test for respiratory viruses (mariPOC® test system, ArcDia International, Turku, Finland) in primary health care.

Methods: Patients with RTI symptoms presenting to a primary healthcare practice in the neighborhood of the Academic Medical Center (AMC) Amsterdam were asked to complete a small questionnaire about his/her symptoms and undergo nasopharyngeal swab sampling. The swab was immediately tested at the point-of-care with the automated mariPOC® test. The mariPOC® test is a simple to perform test for the detection of nine respiratory viruses (influenza A and B, parainfluenza type 1, 2 and 3 viruses, respiratory syncytial virus (RSV), human adenovirus, human bocavirus, and human metapneumovirus) and Streptococcus pneumoniae, with preliminary results ready within 20 min and final results within 2 h. The remaining sample solution was transferred on the same day to the Laboratory of Clinical Virology at the AMC for reference testing with multiplex PCR. Clinical and epidemiological data were collected including age, gender, underlying illness, presenting symptoms, time from onset of symptoms and detected viruses. The sensitivity and specificity of the mariPOC® as compared to PCR was calculated. The clinical feasibility of the mariPOC® test was evaluated using a questionnaire for the study participants and GPs.

Results: From November 11 2015 till March 30 2016 a total of 371 patients (59.3% female, median age 45 years) were included. One or more respiratory viruses were detected by PCR in 43.4% ($n = 161$) of the collected nasopharyngeal swabs. Rhinovirus (RV) was the most frequently detected virus with a prevalence of 11.9%. When reporting samples with Ct up to 40 as positive findings in PCR, the sensitivity and specificity of the mariPOC® test were respectively for influenza A virus ($n = 24$), 54.2% and 98.9%; for influenza B virus ($n = 18$), 72.2% and 99.5% and for RSV ($n = 12$), 50.0% and 100%. In samples with higher viral load (i.e. Ct-value < 30) sensitivity for influenza A, influenza B and RSV was 85.7%, 78.6%, and 87.5%, respectively. The availability of a diagnostic test for respiratory viruses in primary healthcare was appreciated by both patients and GPs.

Conclusion: Respiratory viruses are frequent causes of RTIs in primary health care. Acute infections with high viral loads were accurately detected by the mariPOC test and for these infections a rapid test would be a helpful tool for GPs. Both doctors and patients were positive about the availability of a rapid test in primary health care. The development of a rapid test for rhinovirus would be valuable as rhinovirus was the most frequently detected virus.

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False-negative detection of respiratory syncytial virus as an example that regular update of RT-PCR is required for reliable molecular detection of respiratory viruses

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Objectives: A respiratory sample that was RT-PCR adenovirus positive and negative for other tested respiratory viruses was cultured for adenovirus serotyping in December 2013. Surprisingly, shell vial culture was positive for respiratory syncytial virus (RSV).

Methods: In June 2013 an update of the RT-PCR that was used to detect respiratory viruses was started [1]. The update included the following steps: updating alignments of every target with sequences retrieved from GenBank, amplification and sequence

analysis of primer and probe regions from positive clinical materials, selection and validation of new, updated primers and probes.

Results: Analysis of sequences obtained from GenBank and clinical materials showed that primer and probe sequences for detection of influenza (Flu) A virus, parainfluenza virus (PIV) 1, human rhinovirus (HRV) and human coronaviruses (HCoV) 229E, NL63, and HKU-1 still showed a 100% match to the circulating virus genomes. However, primer and/or probe sequences for detection of Flu B, RSV, PIV2, PIV3, PIV4, human metapneumovirus (HMPV), and HCoV OC43 required some adjustment. The RSV assay, that detects both RSV-A and RSV-B, consisted of two sense primers, one antisense primer and two probes. All sequences obtained from RSV positive clinical isolates from 2013 contained a mismatch to both probe sequences. This mismatch was also observed in two sequences from GenBank (both from The Netherlands, 2012) but not in other L gene sequences from GenBank. Validation of adapted RSV probes was ongoing at the moment that a RSV RT-PCR negative sample resulted in a RSV positive culture. The cultured RSV strain was negative in the diagnostic RT-PCR, but positive in the updated RSV RT-PCR that was validated at that moment. However, the relative fluorescent unit (RFU) signal of the RSV strain was lower than that of positive control material and sequence analysis of the strain showed a mismatch with the new probe. This additional mismatch was not observed in sequences obtained from GenBank, but in January and February 2014 several clinical samples tested in our setting showed RSV signals with low RFU and turned out to have the same mismatch. Therefore, a new update of the RSV RT-PCR was started. Another target of the RT-PCR was considered, but alignment of over 100 whole genomes of RSV showed that the current target region in the L gene remained the target of choice. The two relatively short taqman probes were replaced by a longer taqman probe that should be able to better tolerate mismatches. The new RSV assay will be used next RSV season.

Conclusion: Due to the high mutation frequency in RNA viruses, regular update of RT-PCR assays is mandatory for reliable molecular detection of respiratory viruses.

Reference

[1] Templeton et al., Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4.

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Genetic characterization of human respiratory syncytial virus (hRSV) infecting children in France during two winter seasons

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Background: Worldwide, the human respiratory virus (hRSV) genetic characterization takes a significant place and highlights the importance to monitor the circulation of different genotypes and/or the emergence of new variants. These can affect the susceptibility to the current or future treatments of hRSV infection. There is no information to date regarding the molecular epidemiology of hRSV in France. The aim of this study was to investigate the genetic diversity of group A and B hRSV isolates, obtained from children under 1 year old, during two recent consecutive epidemic periods.

Material and methods: Nasopharyngeal swabs or aspirates obtained from children included in a study who evaluate the efficacy of the use of salt solution 3% in the management of non-complicated bronchiolitis, the "GUERANDE" study, were analyzed. The samples were collected in hospital centers in France who participate to the study during two winter seasons, 2012–2013 and 2013–2014. Viral ARN was extracted using Qiasymphony DSP Virus/Pathogen Mini kit®. All samples were tested by a real time RT-PCR for the detection and group typing of the hRSV A/B. The amplification and sequencing of the second variable region (HRV-2) of the G gene were performed using One-step RT-PCR kit (Qiagen, Hilden, Germany) and specific primers and protocols. The sequences obtained and reference sequences for different genotypes were analyzed with BioEdit® software and phylogenetic tree were constructed by the neighbor-joining method in MEGA7 software.

Results: A total of 719 samples were included in the "GUERANDE" study. These samples were collected from children under 1 year old consulting for a non-complicated bronchiolitis in 24 hospital centers distributed in 12 different French regions. The hRSV group typing identified 375(52.16%) hRSV-A, 247(34.35%) hRSV-B, as well as 14(1.95%) hRSV-A/B co-detections and 83(11.54%) were negatives for hRSV detection. The amplification and sequencing of the HRV-2 G gene were successfully undertaken for 228(60%) of the 375 hRSV-A and 215(87%) of the 247 hRSV-B.

The analyzed sequences of hRSV-A fell within different clusters genotypes, corresponding to ON1 in the majority of cases, but also NA1 and GA2. The ON1 identified sequences were closely related to GA2. The sequences that had been sampled in different epidemics dose not formed distinct clusters.

The phylogenetic analysis of hRSV-B sequences allows the identification of 3 genotypes, BA-9, BA-10 and BA-C. A distinct BA-9 cluster was observed for the sequences sampled in Toulouse. This cluster was confirmed by different phylogenetic analysis. The

BA-C genotype was identified for only 3 sequences sampled in Paris. Concerning the hRSV-B group, in France, the 2012–2013 and 2013–2014 epidemics are homogeneous. The genotypes BA-9 and BA-10 are mainly identified with a predominance of the BA-9 genotype in all regions.

Conclusion: The molecular characteristics of hRSV were determined for the first time in France. The present study describes the genetic diversity of recent circulating strains and contributes with data regarding the respiratory syncytial virus. Subgroup A and B of hRSV were co-circulating with a predominance of ON1 genotype for the hRSV-A and BA-9 genotype for the hRSV-B.

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Measles virus-specific IgG and neutralizing antibodies in South Korean

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Measles is a highly contagious viral disease and causes fever, runny nose, cough and a rash all over the body. Also complications will lead to potentially life-threatening. South Korea declared nation-wide measles elimination in 2006 and verified as measles elimination by the WHO in March 2014. Measles control has a high priority in many countries, and it is important that questions about the possible vaccine failures and waning immunity be addressed so that the strategy to eliminate measles may be evaluated and strengthened.

We investigated measles virus-specific IgG titer and neutralizing antibodies for assessing immunogenicity of measles vaccination in the vaccinated population aged 4–25 years old in Korea. Measles virus-specific IgG antibodies levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits and neutralizing antibodies were determined using plaque reduction neutralization test (PRNT) with Vero/hSLAM cell and Edmonston strain.

In this study, South Korea population's measles immunity is high because of immunization program recommending 2 doses of vaccines after 1997. However measles specific IgG antibody levels and neutralizing antibody levels declined in middle- and high school years. Our data may provide information on measles occurred in vaccinated population.

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Antiviral effect of hydroxyurea on B19V replication

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Parvovirus B19 (B19V) is a human ssDNA virus with a strict tropism towards erythroid progenitor cells in bone marrow. In subjects with underlying haematological disorders or with immune system deficits, the viral-induced arrest of erythropoiesis can lead to severe clinical manifestations requiring hospitalization and supportive treatments. No specific antiviral drugs are presently available for B19V treatment, although recent research showed a specific antiviral activity for the acyclic nucleotide analogue cidofovir. In the search of additional antiviral compounds, hydroxyurea (HU), an S-phase specific inhibitor of DNA synthesis, was evaluated for its possible inhibitory effect towards B19V.

UT7/EpoS1 cell line and peripheral blood mononuclear cells (PBMC)-derived EPCs were infected with B19V, and the activity of HU in the inhibition of B19V replication, expressed as EC_{50}, was assessed by qPCR evaluation of nucleic acids accumulation. Cellular growth, viability and proliferation in the presence of HU was determined in both systems and expressed as CC_{50} values. To characterize the EPCs system, cells were analysed by flow cytometry to evaluate the expression of the specific erythroid markers CD36, CD71 and CD235a, following B19V infection and with or without HU.

B19V replication was inhibited by HU in a dose-dependent manner yielding EC50 values of 139 μM and 175 μM for UT7/EpoS1 and EPCs, respectively. In both systems, the inhibitory effects on cellular replication yielded CC50 values of 457 μM and 491 μM respectively. Flow cytometry analysis indicated that both HU (in the range of the EC_{50} value) and B19V blocked EPCs differentiation along the erythroid lineage, without additive effects.

HU is an approved drug for the therapy of sickle cell disease (SCD). Interestingly, recent data indicate that HU therapy minimizes symptoms of worsened anemia during B19V-induced transient aplastic crisis (TAC) in children with SCD. Our present study provides evidence of an antiviral activity of HU against B19V and lends experimental support for the observed attenuation of clinical symptoms during TAC episodes caused by B19V infection in the SCD population in the context of HU therapy.

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Abstract no: 26
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A modern sub-lingual immunotherapy for neutralization of EBV

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EBV is probably the most spread virus within the human race, because about 90% of the general adult population are carriers of it.

The biological diagnosis is at present codified very well and allows to detect easily the "healthy carriers", those which are normally immunized.

It turns out nevertheless, that those are not necessarily the most numerous, and that there is a large number of patients disturbed by the presence of the virus, and which are going to develop or a

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persistent primary infection or a reactivation, with a lot of different clinical symptoms.

It is well known, that the favourite location of EBV is represented by B-cells on one hand, but also by epithelial and particularly oropharyngeal cells, on the other.

So we developed an immunotherapy administered by sublingual way, as it is already the case in allergy treatment, allowing a close and immediate contact between the virus and the molecular complex for its neutralization.

Besides clinical observation two biological parameters allow us to measure the impact of our treatment – the evolution of the viral serology on one side, – the extent of the virus load on the other side.

In both cases we observe very often an improvement of one and/or the other.

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Evaluation of the antiviral activity of an aqueous extract from *Solidago virgaurea* against Herpes simplex virus type 2

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Herpes simplex virus type 2 (HSV-2) is widely distributed through the human population, infecting more than 500 million people globally [1]. Although typically causing mild diseases this virus may be responsible for severe infections, mainly in immunocompromised patients. Currently, there is a number of systemic antiviral agents against herpesvirus, the most commonly used being acyclovir and related drugs. However, long term treatments with these drugs may result in the development of resistance, especially in immunocompromised patients, which leads to a continuous search for new and better therapeutic alternatives [2]. According to the World Health Organization plants are the best sources for obtaining a wide variety of drugs [3]. So, in the last decades many pharmacological and chemical studies have focused on medicinal plants to the discovery of new natural antiviral compounds.

In the current study the anti-herpetic action of an aqueous extract, obtained by decoction from stems/leaves of *Solidago virgaurea* L. (Asteraceae) was evaluated. Experiments were made in Vero E6 cell cultures infected with HSV-2 and treated with the plant extract at different non-cytotoxic concentrations. Infected non-treated cells were used as controls. Extract cytotoxicity against Vero E6 cells was assessed by the MTT test. The virucidal effect of the extract, evaluated by comparison of the titers of virus particles incubated in contact or in the absence of the extract, proved null. Anti-herpetic activity was investigated by two types of experiments: (1) treatment of infected cells during virus production revealed a mean yield reduction of 94% relatively to non-treated and an IC_{50} of 35.1 μg/mL; (2) treatment of infected cells during virus titration shown a slighter inhibition, but significant size differences between virus plaques formed in treated and control conditions (smaller in treatment conditions). To a further evaluation of the mechanisms that mediate the inhibitory effect of the extract, a kinetic of the first 7 h of infection was performed with and without treatment, to assess possible differences in viral DNA synthesis. DNA samples from infected cells were subjected to PCR with primers that target the viral DNA polymerase gene and PCR products were visualized in agarose gels. Preliminary results showed the expected amplicon both in treated and non-treated conditions. Amplification of viral DNA appears to start after 4 h of infection but, during the period assayed, only increases under the non-treated conditions. This result is consistent with the low inhibition induced by the extract when it is added later than 4 h post-infection. Our results suggest that *S. virgaurea* aqueous extract inhibits HSV-2 replication cycle, if added in the early phase of the infection, possibly by interfering with the viral DNA synthesis.

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Reference

[1] K.J. Looker, et al., Bull. World Health Organ. 86 (2008) 805–812.
[2] J. Piret, G. Boivin, Antimicrob. Agents Chemother. 55 (2011) 459–472.
[3] J.C.O. Sardi, et al., J. Med. Microbiol. 62 (2013) 10–24.

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Ex vivo expansion of human cytomegalovirus specific T cells for adoptive transfer therapy of patients after hematopoietic stem cell transplantation – Optimization of cultivation conditions

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Ex vivo expanded virus specific memory donor T cells can be used in allogeneic hematopoietic stem cell transplantation recipients for prevention of severe disease caused by infections such as HCMV, EBV, ADV, BKV, etc. Phenotype and functional properties of T cell lines are influenced by cultivation conditions. We compared properties of HCMV specific T cells expanded in RPMI 1640 or Cellgro medium with or without addition of 5% human serum (HS), cell growth factors IL4, IL7, IL21 and HCMV derived peptide pools. Expanded cells were characterized by flow cytometry. Functional HCMV T cell response specific for viral antigens pp65, IE1, US3, UL55 was measured by ELISPOT IFN-gamma.

Cultivation in RPMI 1640, 5% human AB serum (CTL medium) for 13 days gave highest T cell expansion rate (4×) and favoured CD4+ T cells against CD8+ T cells. Supplementation with high concentration of IL4 (1666 U/ml) increased expansion rate and IFN-gamma response in comparison with 1000 lower concentration. The expansion rate was slower in serum free Cellgro medium (expansion by 1.8× at day 15) but the latter favoured CD8+ Tcells against CD4+ Tcells. Cultivation in the CellGro medium resulted in high yields of Streptamer HLA B07-RPHERNGFTVL (pp65) positive CD8+ T cells which expanded 120× in comparison with day 0 whereas in CTL medium they expanded 30× only. Phenotype of HCMV Strep+ T cells resembled the phenotype of effector memory T cells (CCR7−,

CD27+, CD45RA−, CD45RO+, PD1−, CD57−). Expansion rate of functional T cells measured by ELISPOT IFN-gamma response was in range 8× to 120× for individual CMV antigens. In the next step, we shall determine cytolytic response against peptide pulsed or virus infected targets and allogeneic reactivity of antiviral T cells expanded in different media. The results will be shown at the meeting.

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False-negative parvovirus B 19 serology in pregnancy. Do we need PCR testing to detect or exclude infection?

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Aim: The aim of the study was to investigate the parvovirus B19 (B19) seroprevalence, new infection and vertical transmission during pregnancy.

Material and methods: Serum samples and questionnaires from 1349 randomly selected pregnant women were included. The samples were randomly selected from a Norwegian Mother and Child Cohort Study (MoBa) that includes 114,000 children and 95,000 mothers recruited from all over Norway from 1999 to 2008. Sera collected around week 17–18 (sample K1) were analysed for B19 IgM and IgG using an enzyme linked immunosorbent assay (Virion/Serion, Würzburg). All samples with IgG positive and IgM negative results in K1 were classified as immune and no further tests were done. IgG and IgM negative samples in K1 or samples with an IgG equivocal result or IgM equivocal or positive result were also analysed for B19 IgG and IgM on the sample taken at birth (sample K2). Mothers with a seroconversion or with IgM equivocal or positive results in K1 or K2 or with IgG equivocal results in K1 or K2 were tested with an in-house real-time B19 PCR. In addition, corresponding umbilical cord blood samples were also analysed with B19 PCR.

Results: Mean age at delivery was 30.4 years and 40.2% was 29 year or younger. Mean gestational age at delivery was 39.7 weeks and 47.1% was nulliparous. Of the 1349 women 61.7% were B19 IgG positive and IgM negative and 36% were both IgG and IgM negative. Among the initially seronegative women 6,8% seroconverted. However, 2,3% had a more inconclusive serological profile including either IgM or IgG equivocal results or IgM positive in K1and/or K2. K1 and K2 samples from these 31 women with inconclusive serological profile and from the 33 women who seroconverted were subjected to B19 PCR. Sixteen (51.6%) with inconclusive serological profile and eighteen (54.6%) of those who seroconverted had virus detectable by PCR either in K1 or K2 or both. Vertical infection was seen in ten (15.6%) of these 64 children. Among the 16 women with inconclusive serological profiles and a positive B19 PCR at week 17-18, seven women were still seronegative also with an alternative B19 IgG assay (Biotrin, Dublin) in the second sample taken at birth (K2).

Conclusion: In this cohort of pregnant women a high incidence (2.5%) of viremic parvovirus B 19 infections was recorded, resulting in 26.5% of the children becoming infected. In almost half of the women with an inconclusive serological profile combined with positive PCR in K1, IgG was negative at birth (K2). In pregnancy parvovirus B19 PCR is thus recommended since serology is often not sufficient to detect or exclude infection.

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Human cytomegalovirus (HCMV) genotyping in congenital infection

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Background: Human cytomegalovirus (HCMV) is the main congenital infection agent, affecting about 0.2–2.2% of all newborns. This pathogen exhibits extensive genetic variability mainly in structural genes encoding envelope glycoproteins. The most relevant is the HCMV glycoprotein B (gB), encoded by the UL55 gene, an important target of the immune system of the human host. On the basis of sequence variation of this gene, the virus can be classified at least into 4 gB genotypes (types 1–4).

Objectives: The aim of this study was to determine the genotypes for UL55, present on samples from congenital and/or perinatal infection cases in Portugal.

Study design: HCMV gB genotyping was performed on 36 HCMV-positive urine samples and 20 amniotic fluid (LA) samples, collected from 2009 to 2016, by real time PCR. To confirm the results, sequencing techniques (Sanger and Next-Generation Sequencing) were performed.

Results: 35 urine samples could be assigned a gB genotype, in 29 was detected a single genotype (13 gB1; 7 gB2; 6 gB4; 3 gB3), and in 6 mixed (>1) genotypes. Of the 19 LA samples, 17 had a single genotype (5 gB1; 5 gB2; 5 gB3; 2 gB4), and in 2 were detected mixed genotypes. No amplification was obtained in the other 2 samples (1 urine and 1 LA). Sequencing techniques did not confirm the presence of mixed infections.

Conclusions: gB1 seems to be the most common genotype in congenital infection in Portugal, consistent with that described in the literature. Also we corroborate the notion that all genotypes can be involved in this type of infection. Mixed infections should be subjected to further analysis, given the apparent contradiction between PCR and sequencing results.

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Abstract no: 36
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Practical experience in laboratory diagnosis of congenital CMV infection over a period of 8 years in Slovakia

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Primary and/or secondary cytomegalovirus infection of pregnant women can lead to congenital (cCMV) or peri-/postnatal infection. Serological screening of women before or during pregnancy for CMV specific antibodies is not usually performed in

Slovakia and there are no data available on the incidence or the long-term outcomes of congenital CMV infections.

For a period of eight years (from April 2008 to April 2016), we retrospectively determined the number of amniotic fluids and dried blood spots (DBSs) analysed for the presence of CMV DNA in our laboratory, as well as the number of cCMV symptomatic neonates born during this time period. In total, 58 amniotic fluids, 23 DBSs and samples from 182 newborns (0–14 days old) with clinical symptoms were analyzed for the presence/viral load of CMV DNA by nested end-point PCR and/or quantitative real-time PCR. CMV DNA was detected in 3 amniotic fluids (2.5×10^3; 1.6×10^5 and 3.9×10^7 IU/ml, respectively), 7 DBSs, and in samples from 18 neonates (whole blood, urine or both).

In the period under review; 84,909 women were examined for syphilis as a part of compulsory screening for infectious diseases in pregnancy. The relatively low number of investigations for cCMV infections demonstrates the lack of awareness about the risks associated with primary and/or non-primary CMV infections in pregnant women.

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Study of enterovirus and parechovirus infections in young children in Spain over a 3-year period

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Introduction: Human enteroviruses (EVs) and more recently parechoviruses (HPeVs) have been recognized as important viral causes of neurological and systemic infections in children. Our aim was to investigate the epidemiology of EV and HPeV infections and their clinical association in young children over a 3-year study period in Spain.

Patients and methods: Prospective and multicentre study (Grant AES PI12-00904) performed in children <3 years of age admitted in 12 Spanish hospitals during 2013-2015. EV and HPeV infections were investigated in cerebrospinal fluids, sera or throat swabs from patients with fever without source (FWS), suspicion of clinical sepsis, meningitis or encephalitis. Clinical data and informed consent were recorded. EV and HPeV detection and genotyping in clinical samples was performed by RT-PCR and further sequencing.

Results: A total of 786 patients were included in the study. The mean age of the children was 5.2 + 9.1 months and 46% of them were neonates (<1 month). Male/female rate was 1.5. Of the 786 samples analysed, 420 (53%) were EV-positive and 45 (6%) were HPeV-positive. 27 different EV types (5 EV species A and 22 EV species B) were identified while all but 3 HPeV were type 3. Overall the 4 types detected most frequently were echovirus (E)-16 (10%), HPeV-3 (9%), E-6 (8%) and E-18 (6%). All HPeV-infected children were <2 months, being their mean age significantly lower than in EV-infected patients (1.7 + 4.9 vs. 5.9 + 10.1 months, $p = 0.008$). The highest incidence of EV infections was between April and July each year, with another small peak in autumn. HPeV-3 circulation was also higher in spring and early summer, but it seems to be biannual. Clinically, EV infections were associated with meningitis (29% vs. 0%, $p = 0.001$), pleocytosis (40% vs. 4%, $p = 0.0006$) and higher leucocytes (10200 + 5000 vs. 7200 + 3800 cells/mm^3, $p = 0.005$) in blood. HPeV infections were associated with irritability (50% vs. 20%, $p = 0.001$), clinical sepsis (33% vs. 6%, $p = 0.0001$), antibiotic treatment (100% vs. 70%, $p = 0.002$), and PICU admission (30% vs. 9%, $p = 0.003$). Both groups had similar proportion of fever symptoms (93% vs. 100%) and exanthema (18% vs. 14%). Only 2 patients had sequelae (1 EV and 1 HPeV-positive).

Conclusions: Significant differences in clinical and epidemiological data were observed between EV and HPeV infections. Different EV types were associated with meningitis (with pleocytosis and leukocytosis) in young children while HPeV type 3 caused more frequently clinical sepsis exclusively in infants less than 2 months of age. Initially the process seems to be more severe in HPeV-infected children, maybe due to the shorter age of the patients, although prognosis is good in general. EVs and HPeVs should be included in the routine screening of samples from young children with neurological or systemic infections to improve their clinical management, preventing unnecessary treatment and prolonged hospitalization.

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Evaluation of viral etiology in central nervous system infections for seven years

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The serious diseases of the central nervous system (CNS), encephalitis and meningitis, has a high fatality and sequele rate especially if it is not diagnosed and treated. Molecular methods of detection, especially PCR, are the tool of choice for viral diagnosis in CNS infections. In this study, viral etiological agents were evaluated in CNS infections suspected with viral etiology in total of routine 3778 tests, done from cerebrospinal fluid (CSF) samples.

Materials and methods: Cerebrospinal fluid samples of patients suspected of viral CNS infections that were admitted to our laboratory between 1.1.2009 and 31.12.2015 for HSV1, HSV2, VZV, EBV, CMV, HHV6 and enterovirus (EV) were evaluated.

Table 1
Nucleic acid test positive CSF samples.

Viruses	*n*	Positive (*n*)	Positivity (%)
HSV 1	1333	24*	1.80
HSV 2	1333	1	0.08
CMV	580	19	3.28
EBV	506	22*	4.35
VZV	216	1*	0.46
HHV6	478	5*	1.05
EV	178	6	3.37

Table 2
Dual etiologic agents detected by nucleic acid test in CSF samples.

Viruses	*n*
HSV1 + EBV	3
HSV1 + HHV 6	1
EBV + VZV	1
EBV + HHV 6	1

Only 487 CSF samples were evaluated for EV by viral isolation (1.1.2009–1.5.2015.) and the remaining 3291 nucleic acid tests (NAT's) were done from CSF samples by real time PCR for the other viruses. Two tests, VZV and EV were done for the last one and five years respectively.

Results: Nucleic acid test positive CSF samples for all of the viruses are shown in Table 1. Dual infections/etiologic agents detected are shown in Table 2. Enterovirus was isolated in 30 (6.20%) of 487 CSF samples by viral culture.

When positives were evaluated viral etiology NAT positive samples were from 44.4% female and 55.6% male patients. Positive samples came mainly from Pediatric, Neurology and Infectious diseases clinics as expected. The high number of positive results were found in samples admitted during December (35.3%), July (12.9%) and November (10.6%). Patient age of positive samples 80% over 18 years.

Conclusion: Positive NAT results obtained in this study with a total of 14.39% are as other reported results world wide being <20% when routine diagnosis is done for all CNS infections. Higher positivity rates are reported for HSV and EV, when only encephalitis or meningitis cases are investigated. Finland's VZV rate of 29% in CNS infections was not observed in this (0.46%) and many other reported studies. Nine of our 19 CMV DNA positive patients were immunosuppressed patients as expected in CMV encephalitis. Dual infections are predominantly seen with EBV and HHV6, that can show a probable cause of encephalitis because of its latency in blood cells/nervous system and ability to reactivate as well as acute infection. Although NAT diagnostic sensitivity for HHV-6 is higher than 95%, positive predictive value is 30% for immunocompetent patients. A wide spectrum approach including other herpes viruses, adenovirus, influenza virüs, arboviruses (like WNV), measles virus, etc., bacteria, fungi, protosoa and helmints with tecniques like microarrays, and multiplexed methods, standardized, quality controlled tests, using increased automation, quantitative approach, supported with evidence based clinical algorithms will improve microbiological definite diagnosis for CNS infections.

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CSF: Is it possible to exclude viral infection by cell count, protein or glucose measurement

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Background: CSF samples are usually submitted for microscopy and bacterial culture but a decision on further testing for the presence of viruses may depend on the presence or absence of elevated white cell counts in the sample. Normal values for total white cells (WCC) in CSF are given as 0–5 in adults, ≤10 in children and ≤20 in neonates. However many studies have reported detecting viral nucleic acid even when cells are absent from the CSF. Further CSF glucose and protein levels may not be reliable predictors of underlying infection nor whether it is viral or bacterial. We undertook a retrospective review of CSF samples submitted for virological testing.

Methods: CSF samples (*N* = 3928) submitted for virological testing in the period from 1/7/2012 to 31/8/2015 were reviewed. All samples were tested by PCR for Herpes simplex virus type 1 (HSV 1), HSV 2, Varicella zoster virus (VZV) and enterovirus, 1644 by PCR for *Streptoccoccus pneumoniae* (Spn), 1451 by PCR for *Neisseria meningititis* (Nmen) and 577 by latex agglutination for cryptococcal antigen. CSF cell counts, glucose and protein levels were extracted from the records if performed.

Results: Of the 3928 CSF samples, enterovirus was detected in 312 (7.9%), VZV in 77 (2.0%) HSV 1 27 (0.7%), HSV 2 29 (0.7%) and in the smaller subsets Spn in 32 (1.9%) and Nmen 36 (2.5%). Mean WCCs were higher in HSV 2 compared to HSV 1 infections and mean protein levels were higher in HSV 2 and VZV than in enterovirus infections. No cells were present in 13.4%, 11.8%, 15%, 8.3% and ≤10 cells were present in 32.6%, 17.6%, 30%, 8.3% of samples from patients with detectable enterovirus, VZV, HSV 1 and HSV 2 infections respectively.

Conclusion: Neither a cell count within the "normal range" nor absence of a pleocytosis are able to exclude the presence of viral nucleic acid within the CSF of patients. Protein and glucose levels are not reliable in excluding viral infections. All samples from patients with suspected meningitis should be tested for the presence of viral nucleic acid irrespective of cell counts.

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Congenital rubella syndrome in a country with high vaccination coverage – Portugal

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Introduction: The teratogenic properties of rubella virus have been discovered in Australia in 1941 by Gregg who associated the occurrence of rubella during pregnancy with the presence of congenital cataracts. A newborn with congenital rubella syndrome may present major or minor malformations or be asymptomatic at birth and later develop clinical manifestations. The introduction of the rubella vaccine in the Portuguese National Vaccination Plan originated a decrease in the number of reported rubella and congenital

rubella cases. Between 1995 and 2008 no cases of congenital rubella were reported in Portugal.

Aim: Evaluation of laboratory results of "probable congenital rubella cases" received for laboratory confirmation at the Portuguese National Health Institute (INSA) between 2009 and 2015.

Material and methods:

Study population: Between 2009 and 2015, a total of 71 children were considered as "probable congenital rubella" cases, and the respective biological specimens were sent to INSA for laboratory confirmation.

Methods:

Laboratory confirmation was performed by RT-PCR for rubella virus RNA detection, and by rubella specific IgM detection.

Results: Seventy-one children were studied between 2009 and 2015. The mothers of 68 children had Portuguese nationality; 2 were from Eastern European countries and 1 from Cape Verde. Seventy children were newborns, and one was 7 months old.

Rubella RNA detection was carried out in several biological products (urine, oropharyngeal exudate and oral fluid) of the 71 children considered as "probable congenital rubella" cases; 68 of these were asymptomatic whose mothers had a rubella positive IgM test at delivery, despite lacking any evidence of a previous rubella infection. The remaining 3 cases evidenced symptoms fulfilling clinic case definition. Two of the mothers reported rash during pregnancy.

The rubella virus RNA was detected in the 3 symptomatic patients, in 2009, 2010 and 2015, respectively and 2 of these patients also presented a positive rubella IgM test. The remaining 68 cases showed a negative IgM test.

The 2009 congenital rubella case was a boy born in Lisbon from an unvaccinated Portuguese mother who was suspected of rubella at 12 weeks of pregnancy.

The 2010 congenital rubella case was a 7 months old child, born and living in Cape Verde. The mother did not mention any kind of rash illness during pregnancy or rubella vaccination.

The 2015 congenital rubella case was a girl born in Lisbon from a Portuguese mother living in Luanda who reported a rubella history during the first trimester of pregnancy.

Discussion: During the last seven years, three cases of congenital rubella were notified in Portugal, two of whom presented *major* malformations and one *minor*.

As to be expected, two of the congenital rubella cases were observed in newborns from symptomatic unvaccinated women.

The 3 SCR cases identified, two were imported from Africa and one was autochthon.

Conclusion: Despite the high vaccination coverage rates Portugal one autochthonous congenital rubella case was notified in 2009 following a 13 years gap. This evidences that rubella prevention and active surveillance cannot be minimized. It is essential to ensure that all women at child bearing age become aware of their rubella immune status and get (re)vaccinated whenever necessary. Vaccination is, to date, the only method available to prevent congenital rubella.

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Herpes simplex virus DNA results of cerebrospinal fluid samples by real-time PCR

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Central nervous system (CNS) infections require a rapid diagnosis and treatment approach due to their potential of rapid progression and the possibility of permanent neurological damage. Herpes simplex virus (HSV) is one of the most common causative microorganisms of acute sporadic viral encephalitis. In diagnosis of CNS infections, the detection of HSV DNA in cerebrospinal fluid by polymerase chain reaction (PCR) is the gold standard method.

In this study, HSV DNA real-time PCR results of cerebrospinal fluid samples (CSF) sent to our microbiology laboratory from patients with prediagnosis of viral CNS infection between 2008-2015 were evaluated retrospectively.

A total of 176 cerebrospinal fluid samples sent to Microbiology Laboratory of Eskisehir Osmangazi University Medical Faculty between 2008 and 2015 were evaluated. After DNA isolation from these samples, HSV 1/2 DNA presence was evaluated with real-time PCR technique by using Artus HSV1/2 RG PCR (Qiagen, Germany) kits on Rotor-Gene system (Corbett Research 6000, Australia). The analytical sensitivity of the kit for HSV1 and HSV2; 120 copies/ml and 160 copies/ml, respectively. Ninety three (52.8%) patients were male and 83 (47.2%) were female, ages ranging from 1 to 74 (median age 36) years. HSV1 DNA was detected by nucleic acid testing in 9 of the 176 patients (5.1%). Five of the 9 HSV1 positive patients were females. Four of the positive patients were from Infection disease department, three of them from Neurology and two were from Anesthesia department.

Even though CNS infections are not rare, the incidence is not clearly established. The early diagnosis of HSV infections is extremely important in central nervous system (CNS) infections due to HSV, as specific antiviral treatment dramatically reduces the mortality. The etiology is unknown in many cases; the development of molecular techniques such as real-time PCR has increased the detection rate of viral agents.

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Abstract no: 276
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DNA detection of herpetic viruses in dried blood spots in children with autism spectrum disorders

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Introduction and aims: Autism spectrum disorders (ASD) are neurodevelopmental disorders without a definitive etiology in most cases. The aim of this study was to evaluate the possible

presence of viruses at birth in children with ASD and in healthy controls.

Materials and methods: The genomes of Cytomegalovirus (CMV), Epstein Barr virus, Herpes simplex virus, Herpesvirus 6-8 and Varicella Zoster virus were investigated in dried blood spots collected at birth from 82 children, 38 with ASD and 44 healthy controls. Extracted DNA was amplified using specific nested-PCR for each herpetic virus.

Results: Cytomegalovirus was the only virus found with a prevalence of congenital CMV infection of 5.3% (2/38) in ASD cases and 0% (0/44) in controls.

Conclusions: The rate of congenital CMV infection was about 10-fold higher in ASD patients than in the Italian general population and this result underlines the importance of the retrospective diagnosis by using DBS-test. Only investigating a wider ASD population it will be possible to determine the real burden of Cytomegalovirus in the evolution of neurodevelopmental disorders.

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Aberrant expression of miR-21, miR-376c miR-145 and their target host genes in Merkel cell polyomavirus-positive non-small cell lung cancer

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Merkel Cell Polyoma Virus (MCPyV) has already been associated with non-small cell lung cancer (NSCLC). In this study, we evaluated expression profiles of miR-21, miR-145, miR-146a, miR-155, miR-302c, miR-367 and miR-376c in MCPyV +ve and MCPyV −ve paraffin-embedded NSCLC tissue samples as well as in MCPyV +ve and MCPyV −ve samples from "healthy" lung tissue. Significant differences were found only in the levels of miR-21, miR-376c and miR-145 in the MCPyV +ve samples compared to the MCPyV −ve tumour samples. Overall, miR-21 and miR-376c expression was higher in tumour compared to healthy tissue samples; however, miR-21 and miR-376c expression was higher in MCPyV +ve compared to MCPyV −ve tumour samples. The expression of target genes of miR-21(*Pten, Bcl-2, Daxx, Pkr, Timp3*), miR-376c (*Grb2, Alk7, Mmp9*) and miR-145 (*Oct-4, Sox2, Fascin1*) and their associated pathways (*Braf, Akt-1, Akt-2, Bax, Hif1a, p53*) was altered between MCPyV +ve tumor samples and their corresponding controls. miR-145 was downregulated in MCPyV +ve compared to MCPyV −ve tumour samples and the corresponding controls. These results show a novel association between miR-21, miR-376c and miR-145 and their host genes with the presence of MCPyV, suggesting a mechanism of virus-specific microRNA signature in NSCLC.

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Establishment and characterization of an in vitro model of human Polyomavirus BK (BKV) infected prostate normal cells

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Introduction: Prostate cancer (PCa) is one of the most common male neoplasm in the Western word, being the most commonly diagnosed non-skin cancer and the second leading cause of cancer death. Various potential risk factors exist for the initial triggering events, including exposure to infectious agents, such as the human Polyomavirus BK (BKV). BKV is a good candidate as risk factor of PCa because it naturally infects the human reno-urinary tract, it establishes latency, and encodes oncoproteins that interfere with tumor suppressors pathways, thus altering the normal progression of cell cycle. The aim of the study is to establish an in vitro model of infection and to investigate the possible co-factorial role of BKV in PCa onset and progression.

Materials and methods: To investigate the potential relationship between BKV infection and PCa, an vitro model was established using the normal epithelial prostate cell line RWPE-1. The titration of the viral load was performed by means of BKV specific-quantitative real time PCR (qPCR) and droplet digital PCR (ddPCR). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed at different time points to evaluate the effect of BKV infection on the growth of RWPE-1 cells. The expression profiles of a panel of 48 cytokines/chemokines were analyzed to identify differences in their kinetics in infected and uninfected cells by multiplex assay. To assess whether BKV infection was able to modify the cells morphology, ultrastructural analysis and analysis of epithelial-mesenchymal transition (EMT) markers in BKV infected, uninfected and cleared cells were conducted.

Preliminary results: RWPE-1 cell line was found to be both susceptible and permissive to BKV infection, reaching a peak of infection after 3 days (3.9×10^6 copies/mL) and the infection lasted for 14 days. The infected cells showed an higher rate of proliferation than the uninfected cells ranging from +37% to +18%. The expression of IL-6, -9 -18 and TNF-α was higher in the infected cells than in the uninfected. Regarding the EMT markers, E-cadherin was expressed in some uninfected RWPE-1 at cell boundaries and upon BKV infection, E-cadherin expression was mainly located in the cytoplasm and in the perinuclear region. Moreover, after infection some big multinucleated cells could be detected. This pattern was maintained also after 30 days post infection.

Conclusions: The RWPE-1 cell line could be used as a model of BKV infection. The viral infection induces molecular and morphological changes in the cells, but the possible cancer progression due to the virus needs to be still elucidated.

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Acute exudative tonsillopharyngitis; Epstein-Barr virus, Herpesvirus and Adenovirus

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Background: Exudative tonsillopharyngitis (ExTP) is a common illness that requires a careful clinical assessment in order to identify underlying etiology and to avoid morbidity and mortality. As a clinical symptom include fever, lymphadenopathy, trismus, dysphagia, pooling of saliva, pharyngeal congestion, erythematous uvula, soft palate erythema, and bilaterally enlarged tonsils covered with white exudates. Tonsillar exudate culture confirmed group A streptococcal (GAS) tonsillitis. Differential diagnosis includes Epstein-Barr virus (EBV), adenovirus, Fusobacteria, *Arcanobacterium hemolyticum*, *Corynebacterium diphtheriae*, *C. ulcerans*, *Francisella tularensis*, *Yersinia enterocolitica*, and *Neisseria gonorrhoea*. Epstein-Barr virus, the most common cause of pseudomembranous tonsillitis, causes tonsillitis with or without infectious mononucleosis. The tonsils can be severely enlarged which are covered with an extensive necrotic, greyish-white membranous exudate. In this study, we aimed to analyse the presence of EBV, Adenovirus and Herpesvirus type- 1(HSV-1) in ExTP.

Material and method: Molecular diagnosis was performed for EBV, Adeno and HSV 1 detection with swab samples from tonsillar membranous exudate of 51 paediatric patients with Exudative tonsillopharyngitis after GAS ruled out (21 men and 30 women, ages between 2 and 16 years) and at the same time, monospot test was performed. DNA extraction from swap samples was carried out from tonsillar membranous exudate, using the Magnesia® Extraction Kit by using the Nucleic Acid Extraction robot (Magnesia® 2448)(Anatolia Geneworks). Bosphore® EBV DNA, ADENO and HSV type 1 Quantification Kits were used for EBV DNA, ADENO and HSV type 1 PCR by Montania® 4896 RT PCR platform (Anatolia).

Results: The frequency of positive EBV DNA cases in the tonsillar membranous exudate in swap samples were 21.5% (11/51). Monospot test was only one of the positive cases in EBV DNA positive. On the side a case of adenovirus, the HSV-1 was detected in two cases.

Conclusions: A meticulous clinical examination would differentiate between the 2 most common causes; Streptococcus and EBV. Adeno and HSV were determined as less causative agents. Streptococcal tonsillitis can be successfully treated with suitable antibiotics. Acyclovir, ganciclovir, and foscarnet have been shown to inhibit EBV DNA polymerase enzyme.

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Epilepsy patients show reactivation of the HHV6 infection

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Background: The human herpes virus 6 (HHV6) is ubiquitous, lymphotropic, immunomodulating, and potentially pathogenic for the neural system beta-herpes virus. HHV6 is associated with such diseases, as multiple sclerosis, mesial temporal lobe epilepsy, and myalgic encephalomyelitis/chronic fatigue syndrome. HHV6 family consists of two different viruses, A and B.

Materials and methods: 53 patients with clinically diagnosed epilepsy and 104 age and gender matched apparently healthy individuals were enrolled in this study. A patient cohort consisted of 66% of females and 34% of males; the mean age was 49 ± 17 years. Presence of HHV-6 viral DNA was analysed, using nested PCR; viral loads was determined by Real-time PCR; HHV-6 variant-specificity was analysed, using restriction endonuclease analysis; U89/90 mRNA presence was detected by RT-PCR; the early (p41) and late (gB) antigens expression was studied by immunofluorescent analysis; levels of TNF-alpha, and presence of HHV-6 specific IgG class antibodies in peripheral blood were examined by ELISA. The GraphPad Prism software (version 6.0) was used for statistical analysis.

Results: The rate of HHV-6 seroprevalence (presence of HHV-6 specific IgG class antibodies in the blood plasma) was similar for the epilepsy patients and healthy individuals (42 out of 53, or 79.2% and 76 out of 104, or 73.1%, respectively). Noteworthy, the HHV-6 DNA in peripheral blood was detected with the significantly higher frequency in patients, compared with the healthy persons (13 out of 53, or 24.5% and 10 out of 104, or 9.6%, respectively, $p = 0.017$). Out of 13 patients, 11 were carrying HHV6B, and only 2 were infected with HHV6A. In all healthy individuals the HHV6B was detected. Furthermore, the significantly higher HHV-6 load (>10 copies/10^6 cells) was detected in patients compared with the healthy individuals (median values were 1574.0 and 131.85 copies/10^6 cells, respectively). The U89/90 mRNA was found in 10 out of 13 patients. The mononuclear cells of the peripheral blood showed the p41 signal in 54.5% of patients, and 45.5% of patients were positive for gB antigens.

The TNF-alpha levels were significantly higher in plasma of patients with an active viral infection (40.09 ± 11.13 pg/ml), in comparison with latently infected patients (18.81 ± 2.52 pg/ml, $p = 0.014$) and with non-infected individuals (7.71 ± 3.07 pg/ml, $p = 0.0001$).

Conclusions: The significantly high frequency of HHV6 presence, the high viral load, expression of the early and late viral antigens, and high levels of pro-inflammatory TNF-alpha allow us to propose that HHV6 infection plays an important role in the development of epilepsy.

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High frequency of JC human polyomavirus in Portuguese wastewaters: A possible source for human infection

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Introduction: JC polyomavirus (JCV) is ubiquitous among the human population worldwide. Primary infection, typically asymptomatic, normally occurs during childhood and is followed by a lifelong persistent infection. JCV is excreted in the urine of nearly half of the infected individuals, without any associated clinical symptom. Under situations of severe immunosuppression, JCV may reactivate and induces a rare but fatal demyelinating disease of the central nervous system known as Progressive Multifocal Leukoencephalopathy (PML). Despite several hypothesis have been raised, the main mode of transmission remains unknown. The high frequency of urinary excretion of JCV lead several authors to evaluate its presence in sewage wastewater systems, though, to date no data have been reported for Portuguese wastewaters. In this order, the present study represents the first comprehensive assessment of JCV in Portuguese wastewaters along with its removal efficiency by wastewater treatment plants (WWTPs), in order to add further information for a possible way of JCV transmission to occur.

Materials and methods: Fifteen WWTP distributed all across Portugal and serving 26.3% of the National population were selected for the present study. Two pairs of influent (WWI) (untreated) and effluent (WWE) (treated) samples, were collected from each WWTP, in September and December 2013, making a total of 60 wastewater samples. Viruses were concentrated by ultracentrifugation as previously described [1], and detection and quantification of JCV DNA in wastewater samples was obtained by a quantitative real-time PCR protocol using a set of four amplification primers and two internal TaqMan probes, previously described [2].

Results: JCV genome was detected in 14 (93%) of the 15 evaluated WWTP, in at least one of the collected samples. Ninety per cent of the tested influent samples revealed detectable JCV DNA, at relatively high concentrations (mean = 5.48 ± 0.74 log10 GC/L). The treatment of wastewater was able to completely remove JCV genome from 14 (52%) of the 27 initial positive assessed WWI samples. In the remaining 13 initially positive WWI samples, despite no complete removal was accomplished, a decrease in JCV concentration was observed in the majority of cases.

Discussion: The ubiquity of JCV infection, claims for a common route of transmission, particularly when seroepidemiological surveys point to childhood as the age for first infection to occur. The present study reveals the consistent detection of JCV genome in sewage from the different regions of Portugal. Moreover, nearly half of the WWTP were not able to completely remove the virus, which, by this manner will end up incorporating treated sewage and be distributed to the surrounding environment. Such viruses may finally contaminate food and water, which may act as vehicles for JCV transmission through oral route.

Reference

[1] A. Rafique, S.C. Jiang, Genetic diversity of human polyomavirus JCPyV in Southern California wastewater, J. Water Health (2008), http://dx.doi.org/10.2166/wh.2008.067.
[2] C.F. Ryschkewitsch, P.N. Jensen, E.O. Major, Multiplex qPCR assay for ultra sensitive detection of JCV DNA with simultaneous identification of genotypes that discriminates non-virulent from virulent variants, J. Clin. Virol. 57 (2013) 243–248, http://dx.doi.org/10.1016/j.jcv.2013.03.009.

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An unusual course of parvovirus B19 infection, strongly suggestive of virus reactivation

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Because of its highly efficient replication in erythroid progenitor cells, parvovirus B19 (B19V) causes an interruption of the red cell production which may result in a more or less severe anemia. After primary infection and elimination of viremia, viral DNA persists at low levels in multiple tissues, probably for life. It is not clear whether the persisting virus is able to reactivate.

We present a case of severe B19V infection in a patient with spherocytosis that was followed by a further episode of severe anemia with B19V viremia three years later. Nearly full-length nucleotide sequencing of the B19V genomes detected in blood during primary infection and after three years revealed complete identity of the genomes. Although not apodictically proven, this case demonstrates that, in rare instances, recurrence of B19V infection might be possible.

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Parvovirus B19: Its real disease associations

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Parvovirus B19 (B19V or primate erythroparvovirus 1, ssDNA) is dependent on actively profilerating erythroid precursor cells for its replication. This particular tropism is determined by the lack of a viral DNA polymerase and by a receptor expressed on eythroid cells, globoside. In addition to the childhood disease erythema infectiosum, as an immunopathological consequence of infection, all relevant pathology brought about by B19V is explained by the property of wiping out erythrocyte precursor cells. A severe breakdown of red cell synthesis or aplastic crisis occurs in cases lacking recruitment from a resting population, like in sickle cell anemia and in expanding fetal erythropoiesis. In the fetus, this anemia

presents as fetal hydrops, without fetal blood transfusion often causing intrauterine death. A temporary arrest of erythropoiesis occurs in all infected individuals, leading to disappearance of reticulocytes. This is usually too short in duration to lead to a decline of hemoglobin levels.

The initial phase of B19V infection is characterized by a brisk viremic peak of an exceptional magnitude, amounting to 10^{12} viral particles/ml or more. This viremia is likely the result of massive replication in the bone marrow. Several studies have found evidence of B19V DNA persisting in a wide array of tissues. This has enabled historical analysis of viral genotypes in skeletal remains. Viral persistence in tissues not known to support viral replication could be understood by less specific uptake processes during the viremic peak and subsequent cellular persistence of DNA, which is not actively degraded.

Independently from these observations in the last decades some additional pathological associations of B19V infection were described. These associations were based on the detection of viral DNA in affected tissues or on the occurrence of viral DNA in blood. The most frequently reported additional association of B19V infection concerns a role in various cardiac disorders, mainly myocarditis and cardiomyopathy. As these findings were described in prestigious cardiology journals, B19V was included among the possible causes of myocarditis. From a virological point of view, this association is difficult to interpret. There is no clear evidence of viral protein expression or an immune response and there are no epidemiological clues of infection, like concomitantly occurring erythema infectiosum. In addition, the nature of cardiac disorders presumably brought about by B19V appears rather variable.

For these reasons, several authors assumed that passive release of B19V DNA of any damaged tissue harboring persisting virus could well be an explanation for the findings. Still, confusion on the possible role of B19V in a number of disorders remains. Recently, in the Sanquin/LUMC research group it was found that simple enzymatic treatment of B19V DNA positive blood samples provided evidence of the existence of two different states: stable, compatible with protected viral particles or vulnerable, possibly consisting of naked DNA. If confirmed, this would enable differentiation of the B19V DNA findings into those directly related to viral replication and those possibly emerging from passive tissue release. This is essential to determine the real disease associations of this infection. In this way, the case of B19V underlines the importance of a plausible pathogenesis before assuming a causal role, solely based on the finding of viral nucleic acid.

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Occurrence, phase and status of human parvovirus B19 infection in patients with myalgic encephalomyelitis/chronic fatigue syndrome

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Introduction: Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is chronic, multifactorial disease with unexplained etiology. Human parvovirus B19 (B19) is immunomodulating single-stranded DNA virus belonging to *Erythrovirus* genus, *Parvoviridae* family, *Parvovirinae* subfamily and is considered as possible pathogen in development of ME/CFS [1,2]. The aim of this study was to estimate frequency and activity phase of B19 infection, viral load, status of infection and clinical symptoms in patients with ME/CFS.

Materials and methods: 200 patients (65% female and 35% male, mean age 38 ± 12 years) with according to CDC criteria diagnosed ME/CFS and 104 age and gender matched apparently healthy individuals were enrolled in this study. Presence of B19 specific IgM and IgG class antibodies were analysed by recomWell and recomLine Parvovirus B19 IgM and IgG kits (Mikrogen Diagnostik). B19 NS1 gene sequence was detected with nested PCR and viral load was estimated by Parvovirus B19 Real-TM Quant (Sacace Biotechnologies) real-time PCR kit.

Results: B19-specific IgG class antibodies were found in 140/200 (70%) patients with ME/CFS and in 60/89 (67.4%) analysed apparently healthy individuals. None of control individuals, though 16/200 (8%) patients with ME/CFS had IgM class antibodies ($p = 0.0038$). Persistent B19 infection in latent phase had 24/200 (12%) patients and 8/104 (7.7%) apparently healthy individuals, whereas in active phase – 34/200 (17%) ME/CFS patients and 2/104 (1.9%) control individuals ($p < 0.0001$). Elevated viral load was detected in 20/58 ME/CFS patients and in none of 10 apparently healthy individuals with B19 infection ($p = 0.0276$). B19 viral load varied from <10 copies to median 1044 (IQR 3180-503.6) copies/10^6 cells.

Analysing B19 specific antibody reaction patterns, results show that 29.3% of patients had infection status after infection (months), 30.7% – past infection (months to years), 12% had status infection long ago, 6.7% – after infection (weeks to months) and one patient had acute infection status. Such typical ME/CFS clinical symptoms as impaired memory, subfebrility, lymphadenopathy and multijoint pain was observed more often in patients with persistent B19 infection in active than in latent phase. In 93.3% of patients onset of symptoms has occurred 8.3 ± 1.7 months ago and in 6.7% of patients symptoms had started before 2.4 ± 0.5 years.

Conclusions: Results demonstrate finding of human parvovirus B19 persistent infection in active phase significantly more frequent and with higher B19 load among patients with ME/CFS than apparently healthy individuals indicating on implication of B19 infection in pathogenesis of ME/CFS. Therefore markers of active B19 infection could be used as one of biomarkers in ME/CFS diagnostics.

Association of persistent B19 infection in active phase with part of typical ME/CFS clinical symptoms shows possible B19 involvement in disease development and reactivation of B19 may be a risk factor for ME/CFS.B19 infection statuses and onset of symptoms allow suggesting the feasible role of B19 infection as a trigger factor for ME/CFS.

Reference

[1] A.S. Bansal, A.S. Bradley, K.N. Bishop, S. Kiani-Alikhan, B. Ford, Chronic fatigue syndrome, the immune system and viral infection, Brain Behav. Immun. 26 (1) (2012) 24–31.
[2] F. Barah, S. Whiteside, S. Batista, J. Morris, Neurological aspects of human parvovirus B19 infection: a systematic review, Rev. Med. Virol. 24 (3) (2014) 154–168.

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Abstract no: 6
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EBV and numerous associated diseases

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EBV is probably the most spread virus within the human race, because about 90% of the general adult population are carriers of it.

The biological diagnosis is at present codified very well and allows to detect easily the "healthy carriers", those which are normally immunized.

It turns out nevertheless, that those are not necessarily the most numerous, and that there is a large number of patients disturbed by the presence of the virus, and which are going to develop or a persistent primary infection or a so-called reactivation.

In both cases aforesaid it is not any more question about some process of infectious nature, but about various disturbances affecting the immune system of some different way in continuation of the interactions with the virus.

The purpose of this to-day presentation is going to show exactly to what extent the EBV is capable, by using the immunitary resources of the patient to favor the development of a large number of diverse pathological disorders, which are involved in the genesis of a set of diseases being responsible of often very different nosological frames, and whose evolution will be favorably influenced by EBV neutralization.

This last one can be realized by applying a therapy with an epigenetic modulation by means of micro-RNAs, those of the virus but of the host-cell too, used within the framework of a sub-lingual immunotherapy.

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Investigation of malignancy associated with EBV (Epstein-barr virus) in paediatric patients with liver transplant

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Background: The aim of this retrospective study was investigation of malignancy associated with EBV (Epstein-Barr Virus) and viral load by real-time polymerase chain reaction (Real-Time PCR) in paediatric patients with liver transplant.

Material and methods: Thirty-two paediatric patients that applied from Gazi University Faculty of Medicine, Department of Paediatric Gastroenterology who were liver transplant between March 2014 and January 2016. EBV VCA IgM, VCA IgG, EBNA IgM, EBNA IgG ve EA IgG antibodies were investigated by Enzyme-Linked Immunosorbent Assay (ELISA) (DIA PRO Milano, Italy) method in clinical samples of patients that required of serological analyses. Serological profiles of patients were determined and EBV DNA was investigated quantitatively by Real-Time PCR. DNAs were extracted by "Spin-Column" method (High Pure Viral Nucleic Acid Kit, Roche, Germany). Isolated DNAs were amplified by Real-Time PCR method in LightCycler 2.0 (Roche, Germany) device and the results were evaluated quantitatively.

Results: In our study 18 females (%56.3), 14 males (%43.7) paediatric patients ages between 1 and 18 years were investigated. EBV DNA was positive for %15.6 (5/32) of patients by Real-Time PCR method. EBV DNA and VCA IgM positivity was not detected simultaneously. The patients that EBV DNA detected were diagnosed with Biliary atresia, Progressive familial intrahepaticcholestasis (PFIC), Wilson, Chronic liver failure and all patients had liver transplantation. There was no rejection in EBV-positive patients and Burkitt's lymphoma developed in one positive patient (1/5). ALT, AST, GGT values were within normal limits in EBV DNA positive patients. EBV DNA positive patients were treated with acyclovir, ganciclovir or val ganciclovir without changing immunosuppressive therapy. One of the EBV positive five patients, have not participated in the treatment process and have not applied again to hospital; one patient died due to Burkitt's lymphoma; for the remaining three patients, it was observed that the process was continuing with therapy and recurrent infections. It is revealed that the most common symptoms are fever, vomiting, diarrhea and tonsillitis in EBV positive patients. EBV DNA was found $\geq 10^4$ copies/ml for the 80% (4/5) of the studied samples and 20% (1/5) had $\geq 10^5$ copies/ml.

Conclusion: EBV infection is a high risk factor in immunosuppressive patients especially transplantation patients, EBV was important for these patients' follow-up and prognosis. Monitoring EBV DNA levels closely with Real-Time PCR methods is helpful for evaluating the changes in the clinical course and early diagnosis. The patient, who was diagnosed with Burkitt's lymphoma, had high viral load ($\geq 10^5$ copies/ml); therefore, in immunosuppressive patients that were detected high levels of EBV DNA, Burkitt's lymphoma disease should be considered.

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