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Abstracts*)

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Plenary Session (A1, A2)

A1

Children with HIV: risks and opportunities

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Without therapy, children with HIV have a 25% chance of dying in the first year of life, with high morbidity and mortality in the majority of older children. The introduction of antiretroviral treatment (ART) has been dramatic, and we now expect most infected children to live to adulthood. However we now face new challenges. Currently, ART is required to be given daily and indefinitely. Maintaining life-long adherence is difficult and the incidence of triple-class virological failure increases with time on therapy. This highlights the urgent need to develop novel strategies to manage HIV in children. HIV-infected infants treated within a few days of birth have the unique combination of a very limited pool of integrated viruses, a very high proportion of relatively HIV 'resistant' naïve T cells and an unparalleled capacity to regenerate an immune repertoire. These features make this the optimal population to investigate the potential efficacy of new approaches to managing HIV. Although less common than in adults, the study of pediatric HIV has been pivotal to our understanding of HIV pathogenesis and management. In this talk, some of the risks of acquiring HIV early in life, and also the opportunities that may exist for HIV infected children now and in the near future will be discussed.

A2

Th-17 cells-microbial translocation, immune activation, and exhaustion in HIV patients

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Background: Chronic HIV-1 infection is associated with disproportionate levels of immune activation and immune exhaustion. Immune activation is associated negatively with immune recovery and positively with progression to AIDS. Persistent immune activation is reported to be consequence of inflammation. However, the association of soluble and cellular markers of inflammation with immune activation and immune exhaustion in the context of immune reconstitution of HIV patients has not been studied.
Objectives: To analyze the association of circulating Th-17 cells (cTh-17) with immune activation (IA) and immune exhaustion (IE) in 20 HIV-1 infected patients with impaired restoration of CD4 T-cell counts despite prolonged suppression of plasma viremia (discordant) and to compare it with 20 HIV-1 infected patients showing good immunologic and virologic responses (concordant) following HAART.
Materials and methods: Twenty HIV-1 infected patients with impaired restoration of CD4 T-cell counts despite prolonged suppression of plasma viremia (discordant) following HAART, 20 HIV-1 infected patients showing good immunologic and virologic responses (concordant) following HAART, and 10 healthy controls were compared. Phenotypic markers were analyzed by flow cytometry; phorbol 12-myristate 13-acetate (PMA) and ionomycin were used to stimulate PBMCs for study of IL-17 production and markers of microbial translocation were quantified using EIA and a limulus amebocyte lysate (LAL) chromogenic endpoint assay.
Results: Discordant HIV-1 infected patients showed significantly higher frequencies of cTh-17 cells compared to concordant patients and healthy controls after PMA + Ionomicin stimulation. Discordant patients also showed higher CD4 T-cell immune activation (HLA-DR+CD38+) than concordant patients which directly correlated with microbial translocation. Additionally, CD4 T-cells of discordant patients showed higher frequencies of CD4 T-cells expressing multiple immune exhaustion markers (Tim3+PD-1+) which correlated with immune activation indicating that combined analysis of inhibitory molecules along with PD-1 might be a better predictor for immune exhaustion of CD4 T-cells. Increased cTh-17 cell frequency correlated inversely with CD4 T-cell percentages and absolute counts and directly with CD4 T-cell immune activation and T-reg frequencies. Persistent CD4 T-cell immune activation might favor differentiation of activated CD4 T-cells towards cTh-17 phenotype in discordant patients. Discordant patients had significantly lower baseline CD4 T-cell counts and higher viral load at the initiation of HAART and higher immune activation and immune exhaustion after being on HAART for long time indicating that these factors might be associated with an increase in cTh-17 cell frequency thus, increasing the risk of disease progression despite virologic control.
Conclusions: The findings from this pilot study suggest that a better understanding of the nature and function of cellular subsets associated with IA and immune regulation in HIV-1 infection may assist in the development of novel strategies for targeting immunosuppression and inflammation in HIV-1 patients on HAART.

Session New Technologies 1 (A3 – A8)

A3

Performance evaluation of the new real-time PCR-based cobas® HCV assay for use on the cobas® 6800/8800 systems for the detection and quantification of HCV RNA

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Background: Quantification of HCV RNA during antiviral therapy is essential for the guidance of treatment duration, to decide on futility, and to determine sustained response to antiviral therapy.
Objectives: To evaluate the performance characteristics of a newly developed Roche real-time HCV RNA assay, the cobas® HCV for use on the cobas® 6800/8800 systems.
Materials and methods: Analytical sensitivity and linearity at lower concentrations (5-1000 IU/mL) were assessed by the cobas® HCV (Roche, Pleasanton, CA, USA) using serum samples representing HCV genotypes (GT) 1-4 and compared with two established FDA-approved HCV RNA assays, the COBAS® AmpliPrep/COBAS® TaqMan HCV Test v2.0 (CAP/CTM) and the COBAS® TaqMan HCV Test for use with the High Pure System v2.0 (HPS/CTM). In addition, pairwise assay comparisons were performed using 245 clinical samples from before, during, and after antiviral therapy. Quantifiable samples within the dynamic ranges of each assay were compared using Deming regression and Bland Altman plot analyses.
Results: The analytical sensitivity of the cobas® HCV was 8.2 IU/mL (95% CI: 6.7-14.4) in samples representing GT 1 and 8.6 (95% CI: 6.6-16.2), 10.4 (95% CI: 7.9-19.0), and 24.6 (95% CI: 18.8-38.8) IU/mL in HCV GT 2, 3, and 4, respectively. The cobas® HCV was mostly linear at lower concentrations with a mean observed log difference of 0.18 in samples containing GT1 (all differences between expected and observed results above the assay limit of detection were <0.3 log). There was high concordance between cobas® HCV vs. CAP/CTM and HPS/CTM, respectively, for the differentiation between detectable and undetectable on-treatment results (n=241/245; 98% and n=239/245; 98%). However, there was some variation in classifying results as undetectable vs. detectable below the limit of quantification (i.e. HCV RNA <15/<25 IU/mL) between the three assays. Pairwise comparison of quantifiable GT 1 samples showed excellent agreement between cobas® HCV and CAP/CTM (mean difference, 0.085; 95% CI: 0.06, 0.11; deming regression equation: cobas HCV= -0.09 x (CAP/CTM) 1.04; R^2=0.99) and cobas® HCV vs. HPS/CTM (mean difference, -0.08; 95% CI: -0.12, -0.04; deming regression equation: cobas® HCV= 0.14 X (HPS/CTM) 0.95; R^2=0.99).
Conclusions: The cobas® HCV is a highly sensitive and linear automated real-time PCR-based HCV RNA assay that compares well to the two established assays, CAP/CTM and HPS/CTM, in samples representing HCV genotypes 1-4 and viral load levels across the linear range of the three assays.

A4

Analysis of drug resistance associated mutations in HCV and HIV using next generation sequencing

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Background: Detection of drug resistance-associated mutations (DRMs) is well established in HIV ART and is increasingly used in HCV patients on treatment with direct acting antiviral agents (DAAs) to detect resistance-associated variants (RAVs). Furthermore, accurate determination of HCV genotypes (GTs) and subgenotypes is still essential. Sanger sequencing has recognized limitations in sensitivity and turnaround time. Next generation sequencing (NGS) provides excellent accuracy, speed, and sensitivity enabling detection of rare mutants, HCV GTs and subgenotypes 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: NGS was used in combination with workflow automation on a newly developed platform based on the epMotion® 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 GTs and subgenotypes and RAVs as well as DRMs in HIV. For HCV, target sequences in the NS3, NS5A, and NS5B regions were employed. For HIV, sequences in the reverse transcriptase and protease regions were selected for NGS.
Results: In an evaluation study including 150 HCV sera, results obtained from HCV genotyping with the new NGS-based assay was compared to those obtained with a line probe assay. Resolution of discrepant results by Sanger sequencing indicated 100% accurate GTs by NGS. Several

results obtained by the line probe assay would have led to a sub-optimal therapy regimen. In an HIV pilot study including 112 plasma samples NGS results were compared to those obtained by TruGene sequencing. The Sentosa® SQ HIV Genotyping Assay detected 100% (199/199) of all mutations in the protease gene and 98.2% (427/435) of all mutations in the reverse transcriptase gene.
Conclusions: Given the crucial role of accurate sequencing analysis in HCV and HIV therapy management, workflow automated NGS appears as a highly reliable tool for differentiating HCV GTs and subgenotypes and RAVs helping to prevent diagnostic errors that may lead to sub-optimal treatment. Considering the pivotal role of DRMs in HIV patients under HAART, the Sentosa® SQ HIV Genotyping workflow appears as a promising 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 detection of HIV DRMs.

A5

Towards standardized monitoring of HDV RNA viral load

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Background: HDV is a small, defective RNA virus that can infect only individuals who have HBV; worldwide more than 15 million people are co-infected. HDV increases the severity of chronic HBV infection, frequently leading to cirrhosis, hepatic decompensation or hepatocellular carcinoma. The prevalence of HDV is declining in some endemic areas but increasing in northern and central Europe because of immigration. Interferon-alpha is currently the only available treatment option leading to suppression of HDV RNA in 25%-30% of patients. However, pegylated interferon therapy is associated with sometimes severe side effects and only a minority of patients is eligible for treatment. Novel alternative treatment options are currently in early clinical trials. Confirmation of HDV infection, as well as standardized monitoring of viral load, is urgently needed in order to personalize patient management. The majority of nucleic assay amplification tests for viral load monitoring of HDV RNA have been developed in-house based on real-time PCR using internal standards of different origin. This issue impedes comparability and was greatly improved with the establishment of the 1st WHO standard for HDV RNA.
Objectives: To evaluate the performance of a novel real-time PCR kit, the RoboGene® HDV RNA Quantification Kit 2.0. To compare with an in-house method.
Materials and methods: Evaluation data achieved according to the common technical specifications for in vitro diagnostic medical devices. Furthermore, samples of 15 HDV IgG-positive (genotype 1) and 5 HDV IgG-negative patients were analyzed at the Medical School of Hannover. The study included a longitudinal perspective as samples were collected before start of therapy and after 12 and 48 weeks treatment with pegylated interferon alpha.
Results: Besides optimal specificity, the assay showed linearity from 5 to 1 x 10^9 copies per run and showed a lower limit of detection of 10 IU/ml. Before start of therapy results obtained by the kit were consistent with those obtained by the in-house method. After 12 weeks treatment, 20% of patient samples were found to be negative with the new kit, in contrast to 33% when using the in-house method. After 48 weeks of treatment, 33% and 40% of patient samples tested negative with the kit and the in-house method, respectively.
Conclusions: The new kit can be reliably used to confirm HDV infection and to determine treatment efficacy. Future studies are in preparation including investigation of kit performance with different HDV genotypes.

A6

Virological monitoring of bone marrow transplant patients

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a standard procedure for several hematological diseases. However, the first year post-transplantation is often complicated by infections and graft versus host disease as consequence of dysregulated immunity. In particular, adenovirus (AdV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), BK polyomavirus (BKV), and human herpesvirus type 6 (HHV6) can cause severe diseases. Therefore, an urgent need exists for better monitoring the immune reconstitution in order to minimize post-transplantation complications. TTV (Torque Teno virus) is another chronically persisting DNA virus and has been reported to be a marker for immune function in transplant recipients. Transient TTV viremia can be detected in >90% of healthy humans.
Objectives: In the present study, the dynamics of TTV viremia was analyzed by real-time PCR in order to define its potential role as marker of immune reconstitution in allo-HSCT recipients.
Materials and methods: For monitoring of the viral load kinetics of AdV, CMV, EBV, BKV, HHV6, and TTV, whole blood samples from 23 allo-HSCT recipients were extracted and assayed with real-time PCR in an automated and multiplexed set-up on the VERSANT® kPCR Molecular

System with MiPLX software solution (Siemens Healthcare GmbH, Erlangen). For quantification of AdV-, CMV-, EBV-, BKV-, and HHV6-DNA loads, VERSANT® PLX assays (Siemens Healthcare GmbH), and for TTV, an in-house developed TTV-DNA assay was applied. TTV viremia was correlated to the blood cell count up to the first 200 days post-transplantation.
Results: All 23 patients showed a detectable TTV-DNA load (100%), in comparison to 31% in immune-competent persons (5/16). The 23 patients showed peak TTV-DNA loads between 107 and 1012 copies/mL up to day 100, in contrast to the low viremia (<104 copies/mL) in controls. By correlating TTV-DNA load to lymphocytes count, patients displayed divergent profiles from day 100 onwards: 14/23 patients showed a reduction or stable state of TTV-DNA load per lymphocytes, while 6/23 showed an increasing viral load per lymphocytes. Three patients were lost to follow-up. CMV was detected in 8/23, BKV in 3/23, EBV in 3/23 patients and were not correlated to TTV-DNA load levels.
Conclusions: HSCT recipients displayed a significantly higher TTV viremia compared to immunocompetent controls. Following the lymphocytes count, TTV-DNA load increased during the first 100 days post-transplantation independently of detectable or not detectable TTV viremia before transplantation. After discontinuation of immunosuppressive agents, the majority of patients showed a decrease of viral load, together with functional reconstitution of the immune system. Overall, dynamics of TTV-DNA load in blood could be considered as an additional parameter to improve immunological monitoring after allo-HSCT.

A7

Molecular point-of-care diagnostics of influenza A and B by lab-in-a-tube testing – the cobas® Liat system

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Background: Rapid detection of influenza A and B is clinically important to start patient directed therapy and to manage infection control measures. Current rapid tests lack sensitivity and molecular nucleic acid tests have relatively long turn-around-times (transport and analysis) and generally require well-trained personnel.
Objectives: To evaluate a molecular point-of-care system, the Roche cobas® Liat system, for the detection of influenza A and B.
Materials and methods: The cobas® Liat, a new FDA-cleared lab-in-a-tube assay allowing combined influenza A and B testing by real-time PCR in less than 20 minutes, was retrospectively evaluated using 113 respiratory samples collected in the 2014-2015 season stored at -20°C. Among the 113 samples, 56 were initially found positive for influenza A and 27 were found positive for influenza B by the routine influenza A and B test (Diagenode, Liege, Belgium). Of the remaining samples, 12 were found positive for a variety of other respiratory viral pathogens and in 18 samples, no viral pathogen was detected by the Diagenode test. The samples were selected randomly and not based on the strength of positivity. Discrepant results were retested by the Cepheid Influenza A/B test.
Results: The cobas® Liat found 51 of the 56 samples positive for influenza A. The 5 discrepant results were repeated with the Cepheid Influenza A/B test, which confirmed 2 positive cases (with very low viral loads). Three influenza-positive cases by the Diagenode test (Ct values: 39.05, 38.51, and 40.00) were found to be negative with the Cepheid test as well. All 27 influenza B-positive samples by the Diagenode test were found to be positive by the Liat test. None of the samples positive for non-influenza pathogens were found positive by the Liat and in all of the 18 negative samples, no influenza A or B was detected by the Liat as well. Therefore, the cobas® Liat showed a sensitivity for the detection of influenza A and B of 96% and 100%, respectively, with a specificity of 100% for both targets.
Conclusions: The cobas® Liat system is a very rapid, sensitive, specific, and easy to handle molecular system, which can be used in a point-of-care setting enabling timely and tailor-made patient management and infection control.

A8

Multiplex detection of respiratory pathogens with GenMark's ePlex™ sample-to-answer system

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Background: Respiratory tract infections continue to be a persistent health concern and are among the most common causes of morbidity and mortality worldwide. Traditional diagnostic methods, such as culture and serology, are being replaced with more advanced molecular techniques with faster turnaround times and greater sensitivity and specificity. GenMark Diagnostics is developing a Respiratory Pathogen (RP) Panel targeting 20 viral and 4 bacterial targets commonly associated with respiratory infection, which can be used on the ePlex™ sample-to-answer system. The ePlex™ system incorporates sample extraction, RT-PCR amplification, and eSensor® electrochemical detection technology in a single-use, self-contained cartridge. The RP Panel is designed for optimal workflow and throughput from sample to result in about 90 minutes with less than 2 minutes of hands-on time.

Objectives: To evaluate performance of the ePlex™ RP Panel with nasopharyngeal swab (NPS) samples and to compare to results from other commercially available respiratory tests.
Materials and methods: A total of 198 clinical NPS samples were retrospectively tested by the GenMark ePlex™ RP Panel and results were compared to the original test results obtained by either the BioFire FilmArray RP (92 samples) or GenMark XT-8 RVP (106 samples). Tests were performed according to manufacturer's instructions. All discordant samples were confirmed with either XT-8 RVP or sequencing.
Results: Results showed the ePlex™ RP Panel was 98% concordant with FilmArray RP, upon resolution of discordant samples. ePlex™ RP missed 2 parainfluenza virus type 3 (PIV-3) targets compared to FilmArray RP, and FilmArray RP missed 3 targets that ePlex™ RP detected, including adenovirus C, influenza B, and PIV-4. ePlex™ RP versus XT-8 RVP results showed 100% concordance for 9 viruses and all negative samples. Four samples were positive for rhinovirus and 4 were positive for other viruses (single infection), as detected only with XT-8 RVP upon discordant resolution.
Conclusions: Results from this study demonstrated high analytical sensitivity with the ePlex™ RP panel and confirmed the ease of use of the ePlex™ sample-to-answer system.

Session New Technologies 2 (A9 – A14)

A9

Efficient processing of complex test orders with an automated molecular diagnostics system

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Background: Molecular diagnostics laboratories are increasingly confronted with complex test orders including more assays in smaller sample sets and diverse sample types. The overhead and lack of flexibility often associated with existent automation solutions can render automated processing of these test orders inefficient with regard to cost and time.
Objectives: To establish a system allowing the automated processing of complex test orders avoiding the drawbacks often associated with automation. To integrate the altona Diagnostics assay menu in this automation system.
Materials and methods: The CE-IVD certified hardware was combined with custom-made workflow software. The configuration of assay and purification kits was adapted to leverage the full functionality of the system.
Results: The molecular diagnostics system was shown to perform automated, combined purification of 96 plasma, whole blood, urine, stool, swab, and cerebrospinal fluid samples with a single purification kit, automated PCR setup combining these purified samples with up to 8 different assays, and automatically programmed real time PCR. Waste of reagents was minimized irrespective of sample set size. The sample and process information was passed through the complete process to achieve full traceability and rule out misinterpretation of results.
Conclusions: Complex test orders can be processed efficiently with regard to cost and time with an automation system that facilitates the combination of different sample types with a broad range of diagnostic assays, avoids waste of reagents, and optimizes the workload of the hardware modules.

A10

Flexibility and full automation for clinical sample extraction – preliminary performance evaluation of the new bioMerieux eMAG®

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While molecular testing continues to play an increasingly important role in human diagnostics, molecular laboratories are confronted with numerous challenges resulting from more comprehensive test menus, consolidation of laboratory testing, 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. BioMerieux's NucliSENS easyMAG® has been the preferred choice by many laboratories worldwide due to its flexibility and adaptability to individual needs, but it requires several manual preparation steps and provides limited throughput. In this talk, preliminary results of the performance evaluation of the new bioMerieux eMAG® which provides full automation of sample extraction starting from primary tubes

and using well established easyMAG® chemistry is presented. Higher throughput, process traceability, and seamless integration into the diagnostic laboratory workflow have been primary design goals for this next generation platform. Different workflow options and first evaluation results from laboratory studies will also be presented.

A11

Meet the diagnostic algorithm with modular tests – Zika virus included in the tropical panel

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Background: Pathogen testing depends on clinical symptoms, season, and region. Test panels must be adapted in case of an emerging disease. A modular test design allows fast adaptions of the test menu. Explosively increasing numbers of Zika virus infections have been reported for several South American countries since end of 2015. Symptoms are similar to Dengue infections.
Objectives: To establish a panel for simultaneous detection of Chikungunya, Dengue, and Zika viruses according to the present WHO recommendation.
Materials and methods: The Zika virus test was added to the ModularDx Tropical panel designed for the Roche LC480 instruments. The new Zika virus test targets the NS2A region. It was compared with published Zikavirus RT PCR tests, using the reference strain, African, and South American virus isolates. The new panel includes tests for detection of Chikungunya, Dengue, and Zika viruses and includes a control assay.
Results: The new modular panel performed well in a multicenter study including laboratories in South America, Africa, and Oceania.
Conclusions: The new tetraplex assay allows a rapid adaption of the test algorithm, in particular during outbreak situations and includes testing for pathogens producing similar symptoms.

A12

Preanalytical requirements for the analysis of free circulating mitochondrial DNA

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Background: Free circulating nucleic acids (fc-NA) in the peripheral blood are promising biomarkers for cancer and other diseases with increased tissue turnover and thus associated with increased release of nucleic acids from the cells. For several reasons, among fc-NA, mitochondrial DNA (mtDNA) appears particularly well suited as biomarker: mtDNA has an approximately 100-fold higher mutation rate than nuclear DNA (nDNA), almost all known tumors have DNA mutations, the mitochondrial genome with its only 16.6 kilobases can be easily sequenced in its entire length and the molar concentration of mtDNA is 100 to 1000 times higher than the concentration of nDNA. Despite these advantages, the analysis of free circulating mtDNA (fc-mtDNA) could not find its way into routine diagnostics yet. An important reason for this could be the insufficient plasma preparation in hitherto performed studies. For the purpose of reliable fc-mtDNA analyses, this step must not only ensure the complete removal of leukocytes and erythrocytes, as sufficient for most biomarkers including free circulating nDNA, but also of platelets and (mainly platelet-derived) microparticles, whose mtDNA content likely exceeds the quantity of real free circulating mtDNA by several orders of magnitude. Various plasma separation protocols including one or two centrifugation and sometimes additional filtration steps have been used in published studies on fc-mtDNA, however, to the best of our knowledge, no systematic evaluation of their respective abilities to completely remove mtDNA-containing platelets and microparticles has been reported to date.
Objectives: To establish a standardized protocol for plasma separation for fc-mtDNA investigation purposes. To reduce platelet and microparticle contamination to undetectable levels.
Materials and methods: Plasma was prepared by either 1 or 2 short centrifugations (at 1,600 and 16,000 g, respectively). Blood samples from 10 donors were subjected to centrifugation and further purified by 2 additional filtrations (0.8 and 0.2 μM, respectively) and a final ultracentrifugation (100,000 g for 1.5 h). Platelets were counted and nDNA as well as mtDNA were determined by qPCR after each purification step.
Results: Both of the protocols employed did not ensure the complete removal of platelets. The nDNA was not reduced by further purification steps following the initial short centrifugation at 1,600g. In contrast, the mtDNA decreased by a factor 100 following the second centrifugation at 16,000 g and by a further factor 100 after the additional 3 purification steps of this protocol.
Conclusions: The 5-step protocol described here should be the minimal standard for future fc-mtDNA studies. The residual degree of contamination by very small microparticles even after its completion is subject to future investigations.

A13

Non-invasive prenatal testing by digital counting of fluorescently labeled DNA molecules

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Background: Several countries have implemented *Non Invasive Prenatal Testing* (NIPT) to analyze chromosomal abnormalities in high-risk pregnancies. The majority of these tests are performed using next generation sequencing (NGS) technologies that provide both superior specificity and sensitivity compared to traditional first trimester screening. However, in order to provide all women with high performance prenatal screening, the NIPT assay cost and complexity need to be dramatically reduced.
Objectives: To present a platform that reduces the cost of the analysis by an order of magnitude making the assay available for high throughput diagnostic laboratories.
Materials and methods: The Smart NIPT technology provides a very high assay precision by counting individual fluorescently labeled DNA molecules. The Smart NIPT technology uses DNA probes to specifically convert chromosomal targets of interest into DNA circles. These circles are then clonally expanded into discrete fluorescently labeled DNA objects. The DNA objects are immobilized on a transparent nanopore filter and finally imaged and counted through the bottom of the well. By capturing thousands of DNA targets from each chromosome, PCR amplification can be avoided with increased assay precision and reduced contamination risks as a result. The new technology was applied to analyze 183 blinded plasma samples of which 15 were from women pregnant with a trisomy 21 fetus.
Results: All positive samples were classified correctly, separated from the normal samples with a minimum of 6.6 standard deviations. No false positives were called.
Conclusions: The Smart NIPT platform, supported by convincing clinical data, is a new solution that holds promise to provide NIPT to all pregnant women.

A14

Development of a new multiplex-based Real-Time RT-PCR assay for comprehensive detection of the four most common BCR-ABL breakpoint mutations

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Background: The BCR-ABL oncogene is formed by reciprocal translocation of the ABL gene on chromosome 9 and the BCR gene on chromosome 22. A fusion protein is thus generated and despite of the chromosomal rearrangement, the kinase domain of the ABL protein still remains intact and even constitutively active thereby driving deregulated myeloproliferation of mature granulocytes and their precursors. This situation causes chronic myeloid leukemia (CML) which comprises 15-20% of adult leukemia. While the chromosomal breakpoint in the ABL gene is usually located on exon 2, the breakpoint of the BCR gene can be located on different exons. The breakpoints of the two predominant fusion transcripts *b2a2* and *b3a2* coding for the fusion protein *p210* are located on exon 13 and 14, respectively. However, two more infrequent breakpoints are present on exon 1 (*e1a2*) and exon 19 (*e19a2*) of the BCR gene leading to the fusion proteins *p190* and *p230*, respectively.
Objectives: To develop an assay for detection of the four most common BCR-ABL translocations in a single reaction with the following characteristics: fast, simple, robust, sensitive, specific, and reproducible. Furthermore, cost should be reduced by minimizing reaction set-up and it should be possible to establish the test procedure easily in different laboratory environments.
Materials and methods: Multiplex amplification by real-time RT-PCR was done using a one-step RT-PCR mastermix containing SYBR green. The primer sequences were optimized for a single multiplex reaction. BCR-ABL translocation was detected by a specific hydrolysis probe and identification of the fusion transcript was accomplished by melting curve analysis. A region of the ABL gene was amplified as internal control. Artificial DNA oligos were tested and interlaboratory comparison studies were performed.
Results: Amplification products of fusion transcripts and the internal control were successfully generated showing distinct peaks in the melting curve analysis. Proper function of the assay was confirmed on different real-time PCR cyclers with plate or rotor formats.
Conclusions: A real-time RT-PCR assay was developed that allows detection of the four main BCR-ABL fusion transcripts and an internal control gene in a single multiplex reaction. This was made possible by combination of the intercalating dye SYBR green and a specific hydrolysis probe. Further evaluations of the assay with clinical specimens are in progress.

Session Pathogens 1 – HCV, HIV (A15 – A18)

A15

Hepatitis C virus kinetics in the era of direct antiviral agents

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Hepatitis C virus (HCV) kinetics has long been used as an important clinical tool to guide anti-HCV treatment duration. Response-guided treatment became a central paradigm for treating patients after the approval of the first HCV protease inhibitors in 2011. With the addition of boceprevir and telaprevir to the pegylated interferon/ribavirin backbone, a more rapid and profound virus reduction could be achieved. Patients who clear the virus from their bloodstream within the first four weeks are eligible to receive a shorter duration of therapy and this rule could be applied in the majority of treatment-naïve HCV genotype 1 patients. For both, boceprevir and telaprevir-based therapies, use of highly sensitive, real-time PCR-based assays are required to identify rapid responders who can receive shortened treatment duration and to predict futility. More recently, approval of several direct antiviral agents (DAAs) delivered the long-awaited reality of interferon-free therapies that are both highly efficient and well tolerated. Given the rapid viral load reduction that is seen with most DAA regimens as well as the overall high response rates, HCV RNA kinetics have been shown to be of limited clinical use. However, assessment of viral load at very early time points may play a role in more difficult to treat patients (e.g. those infected with HCV genotype 3) and in those receiving ultra-short therapy. The implications of response-guided therapy in the era of all-oral DAA therapies will be discussed.

A16

Hepatitis C virus (HCV) screening project of patients on anti-HCV therapy

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Background: Clinical outcome of anti-hepatitis C virus (HCV) therapy with direct acting antivirals (DAAs) is still influenced by host and viral factors.
Objectives: To collect data about resistance-associated-mutations (RAMs) within the viral NS3/protease, NS5A, and NS5B genes, frequencies of viral variants, and host factors in an observational, retrospective, and non-interventional study.
Materials and methods: NS3/protease, NS5A and NS5B sequences from baseline (BL) samples were obtained. Subtyping and resistance against Asunaprevir (ASV), Boceprevir (BOC), Grazoprevir (GZV), Paritaprevir (PTV), Simeprevir (SMV), Telaprevir (TVR), Daclatasvir (DCV), Elbasvir (ELV), Ledipasvir (LDV), Ombitasvir (OBV), Dasabuvir (DSV), and Sofosbuvir (SOF) was determined by sequencing (Sanger or Next Generation) and subsequent interpretation of the corresponding viral gene with geno2pheno[HCV] (www.geno2pheno.org).
Results: 992 NS5B sequences were (sub)genotyped: 40.8% ST1a, 36.6% ST1b, 0.2% ST1d, 2.8% GT2, 14.2% GT3, 5.3% GT4. BL NS3/protease-inhibitor susceptibility was determined from 446 BL sequences and used for BL resistance prediction. BL resistance mutations were found for ASV (3.0%), BOC (8.8%), GZV (0.6%), PTV (2.8%), SMV (23.2) and TVR (3.0). For TVR, 20.9% of the samples were predicted as possibly resistant. BL NS5A-inhibitor susceptibility was analyzed for 251 sequences. The percentage of samples with BL resistance mutations was similar for each of the four available NS5A-inhibitors (4.4-6.0%). BL NS5B-inhibitors susceptibility: The sequence sets used for the analysis of each of the NS5B inhibitors varied, since the described RAM-patterns for each drug comprise different amino acid residues (which were not included in all the PCR products of the analyzed samples). While 10.8% of the 278 sequences used for DSV screening were reported with resistance mutations, none of the 742 samples screened for SOF was predicted as resistant for SOF. BL RAM analysis: The mutation NS3 80K

(resistance to SMV) was found in 20.0% of the samples. Substitutions on additional four amino acid positions were found in ≥0.9% of the samples. In the NS5A, not only primary resistance mutations were found but also amino acids exchanges most frequently at positions 30 and 93 showing a high degree of cross resistance. In the NS5B, the mutations 556GNR were found in 12.0% of the cases impairing one of the two available NS5B inhibitors.
Conclusions: Resistance testing for anti-HCV therapy is feasible, especially with using a freely available internet tool. A remarkable number of RAMs in baseline sequences was found. Baseline sequencing prior to anti-HCV therapy with DAAs may help to support personalized therapy decisions.

A17

Screening for acute HIV infection: algorithms and cost-effectiveness

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Background: As many as half of HIV transmissions occur from persons with acute HIV infection (AHI), which makes detection of AHI critical to HIV prevention strategies. However, screening for AHI is not widely performed in community-based settings. Assays that reliably detect AHI require follow-up for results and are generally more costly to perform. By comparing four community based testing strategies, we have recently shown that costs for detection of one case of AHI may be below US $20,000 in at risk men who have sex with men (MSM) (1). Calculation of cost-effectiveness per transmission prevented (i.e. infection averted [IA]) is more complicated, but has important advantages including cost thresholds that are easier to define.
Objectives: To evaluate the cost-effectiveness of field-based AHI testing algorithms compared to HIV antibody (Ab) testing alone on potential of averting new infections.
Materials and methods: We evaluated annual costs of four HIV testing strategies; including three that detect AHI (based on HIV nucleic-acid-amplification testing in all Ab negative persons or HIV p24 antigen detection). These AHI algorithms were compared to point-of-care HIV Ab-testing alone. The cost model had a one-year time horizon and focused on men who have sex with men (MSM). Data sources for model parameters included actual cost and prevalence data derived from a community-based AHI screening program in San Diego, and published studies. Incremental cost effectiveness ratios were calculated by comparing each two testing algorithms, with the numerator representing the differences of annual costs of the two algorithms and the denominator representing the difference in IA. The lower end of the cost range of discounted lifetime costs of an HIV infection (i.e. $229,800) was used for defining cost-effectiveness. Probabilistic sensitivity analysis (PSA) was performed for all input variables.
Results: Our results suggest that all three algorithms that detect AHI were cost-effective versus HIV antibody testing alone. The most sensitive algorithm for AHI detection, which was based on HIV nucleic acid amplification testing, was estimated to prevent between 5 and 45 transmissions, with simulated costs per infection averted between $965 and $141,256 when compared to HIV antibody testing alone.
Conclusions: These results indicate that community based AHI testing among MSM in the United States can pay for itself over the long run.

A18

HIV and cardiovascular risk in a study population of mixed ancestry living in Cape Town, South Africa: preliminary data from the EndoAfrica study

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Background: It is well established that HIV-infection and some antiretroviral treatment (ART) drugs are associated with increased cardiovascular risk; however, most studies are conducted in developed countries. Conversely, there is a paucity of data from sub-Saharan Africa (the epicenter of the global HIV/AIDS epidemic), which is reason for concern, as the population demographics, socio-economic conditions, HIV-1 strains and ART drug combinations are different compared to developed countries of Europe and North America.
Objectives: To assess whether an association exists between HIV-infection, ART and cardiovascular risk in a population of mainly mixed ancestry in Cape Town, South Africa.
Materials and methods: A cross-sectional population study involving participants (HIV-negative and HIV-positive, with or without ART) recruited from health care facilities between August 2015 and February 2016. Traditional cardiovascular risk factors were assessed, as well as vascular and endothelial function by means of flow-mediated dilatation (FMD) and retinal microvascular caliber measurements.

Results: The total sample size was 65 (HIV-negative: 22; HIV-positive: 43). The majority of participants were female (75%), of mixed ancestry (78%), and smokers (58%). Fourteen HIV-positive participants were classified as ART-naïve (HIV/-ART), and 29 were receiving ART (HIV/+ART) (treatment duration 4.53±3.53 years). The mean viral load (RNA copies/ml) in the HIV/-ART group was 3418 (310-64486) and 253 (20-2128) in the HIV/+ART group (p=0.008). Similarly, the CD4 counts (10^6/l) were 343±155 (HIV/-ART) and 517±271 (HIV/+ART), respectively (p=0.03). HIV-negative participants had higher BMI, waist-hip-ratio, and systolic and diastolic blood pressure values compared to HIV-positives but lipid profiles were similar. Both systolic (r = -0.45; p=0.04) and diastolic blood pressure (r = -0.49; p=0.02) correlated inversely with viral load. There were no differences in vascular endothelial measurements between the HIV-negative and -positive groups; however, HIV/-ART participants showed increased baseline brachial artery shear rate vs. HIV/+ART and HIV-negatives (p<0.05).
Conclusions: The data suggest that the traditional cardiovascular risk factors overweight/obesity and hypertension were more prevalent in the HIV-negative control group. There was no evidence of vascular / endothelial damage in any of the groups, and although baseline shear rate was increased in untreated HIV participants, this did not result in changes in endothelial function.

Session Pathogens 2 – HPV, Respiratory / Gastrointestinal Viruses (A19 – A21)

A19

HPV and p16/Ki-67 biomarker testing in routine: correlation with histology

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Background: In Germany, an S3 guideline will be released soon recommending primary HPV screening in cervical cancer prevention while a new obligatory guideline will introduce primary HPV screening alternatively to cytology within the next years. On the other hand, doubt has been raised recently regarding sensitivity of high-risk HPV testing to detect invasive cervical cancer. Meanwhile, p16/Ki-67 (Cintec Plus®, Roche Ventana, Mannheim, Germany) has become the best validated biomarker in cervical cancer screening.
Objectives: To correlate the HPV and the p16/Ki-67 status of patients with CIN (cervical intraepithelial neoplasia) 2+ tested under routine conditions before biopsy and/or therapy (executed maximally six months earlier) with the respective histology results.
Materials and methods: All cases of a German commercial laboratory in which histology was performed within the year 2013 and results of DNA tests for HPV high-risk types and/or p16/Ki-67 analyses were available were included. All tests were done by using cervical smears according to the manufacturers' instructions. 55.0% of HPV tests were performed with the HC2 test (Qiagen, Hilden, Germany) and 45.0% with the cobas® test (Roche Diagnostics, Mannheim, Germany).
Results: In 78.1% of 1004 histologically confirmed CIN 2+ cases, an HPV result and in 57.6% a p16/Ki-67 result was available. 737 (97.4%) CIN 2 and 3 were positive and 20 (2.6%) negative for HPV. The percentage of positives and negatives did not differ between CIN 2 and 3. 25 of 27 (93%) invasive cervical carcinomas where an HPV result was available were HPV-positive while two (7%) were HPV-negative. Taking into account the test system, 2.3% of HC2 and 3.4% of cobas® tests were negative in CIN 2+. In CIN 2, 108 (92.3%), in CIN 3, 443 (100%), and in cervical cancer, 18 (100%) were p16/Ki-67-positive. Among the 15 HPV-negative CIN 2 and 3 cases where p16/Ki-67 tests were performed, 14 (93%) were positive.
Conclusions: The large majority of histologically confirmed CIN 2+ was HPV-HR- and p16/Ki-67-positive when tested in cervical smears taken less than 6 months before biopsy and/or therapy. A high percentage of the rare HPV-negative cases were positive for the biomarker p16/Ki-67. These results from routine testing point to a high sensitivity of high-risk HPV and p16/Ki-67 testing for CIN 2+ and especially for invasive cervical cancer.

A20

Point-of-Impact testing for respiratory viruses in the emergency room

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Laboratory tests based on molecular techniques are increasingly crucial in clinical settings regarding infectious diseases. New (commercial) diagnostic tests do result in a shift from single to multiplex testing and possibly from centralized to decentralized Point-of-Impact (POI) testing. However, due to the critical window of opportunity for innovations such as POI testing, the objectives of our study were to assess the value of POI testing regarding respiratory viral infections and to improve diagnostic policy regarding turn-around-time, cost, treatment, and

infection control. This proof of concept was evaluated at the emergency department of a large tertiary hospital using the BioFire® FilmArray® (bioMerieux). Data on turn-around-time, a model for presenting cost/benefit, and changing the mindset of health care professionals, will be discussed.

A21

Intra-individual noroviral evolution analyzed by next generation sequencing

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Background: Noroviruses have become an important cause for infectious gastroenteritis. Chronic infections have been described recently and will become more prevalent with increasing numbers of immunocompromised patients.
Objectives: To study noroviral evolution in 8 patients analyzing 27 samples. Longitudinal specimens covered 38–470 days.
Materials and methods: Amplicons comprising 760 nucleotides and labeled with barcodes on both ends served as templates for sequencing. Consensus sequences of all samples were used for genotyping and multiple sequence alignment, including phylogenetic analysis. Quasispecies were reconstructed with the software QuRe.
Results: About 80% of the reads could be uniquely aligned to our reference strain Norovirus Hu/GII-4/Aichi1/2008/JP. All consensus sequences were categorized as genotype II.4 strain, 2006b variant. Variable sites between consensus sequences corresponded to previous reports and putative epitopes. The evolutionary rate diversified over time and was higher on amino acid level. Number of reconstructed quasispecies differed widely between samples. They increased at the onset of infection and decreased before cure in 2 patients with the last documented noropositive sample available. Most reconstructed quasispecies were negatively or neutrally selected, some quasispecies of one patient with cured infection were positively selected according to dN/dS analysis.
Conclusions: Fast intra-individual evolution was observed in chronically infected patients, most likely driven by immune selection. Quasispecies varied but increased at the onset of infection and ultimately decreased before cure.

Session Pathogens 3 – HDV / Transfusion-related Pathogens (A22 – A24)

A22

How reliable is HDV RNA quantification?

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Background: HDV RNA quantification is a crucial tool to diagnose, treat, and manage HDV infections. As most assays for detection of HDV RNA are not standardized, comparison of tests results is difficult. The WHO endorsed a project for development of the First International Standard (IS) for HDV RNA (genotype 1). The standard was established by the Expert Committee on Biological Standardization of WHO in October 2013.
Objectives: To demonstrate the commutability of the IS.
Materials and methods: Different EQA and performance studies (PEI, QCMD) were initiated. The study designs included testing of HDV positive clinical samples alongside with the IS. The PEI multicenter study (4 sites) included a 12-member panel consisting of 10 different neat HDV positive plasma samples and two replicate samples of the IS. The QCMD HDV pilot study included a 10-member panel consisting of tenfold dilutions of two genotype 1 HDV clinical samples and the IS, and one HDV negative plasma sample.
Results: Four different quantitative real-time NAT assays were included in the PEI study. The results were analyzed by pair-wise test comparison. Results obtained by the HDV clinical samples harmonized very well within two assays and the IS results fitted within the CI of the regression line demonstrating high commutability. The results of the other two NAT assays showed less correlation compared to the results of the first two assays due to strong under- and overestimation, respectively. The first QCMD HDV pilot study was performed in 2014. Overall,

43 laboratories participated in the study (23 data sets for qualitative NAT assays and 20 datasets for quantitative NAT assays). Qualitative test results showed a sufficient performance in detecting of HDV RNA positive samples up to 1,000 IU/ml. An issue regarding the analytical test sensitivity was observed as only 15 out of 23 laboratories were able to detect a sample representing a concentration of 100 IU/ml. Comparison of quantitative results revealed remarkable differences of absolute concentration values (test-specific copies/ml) of study samples between NAT assays which can be traced to the test design and insufficient calibration. But the majority of the tests showed a sufficient intra-assay correlation of the HDV RNA concentration between clinical samples and IS samples.
Conclusions: Study results demonstrate the commutability of the IS. A careful assay design and test calibration using the IS as a common reference material is needed to achieve reliable HDV RNA detection and quantification.

A23

Estimation of residual infection risks in blood components

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Background: On a global scale, an estimated amount of 9.3 million liters of recovered human plasma is discarded annually which, if fulfilling several quality requirements and preconditions, could be used for the manufacture of plasma derived medicinal products (PDMPs). At the same time, respective countries import PDMPs to address the needs of their population.
Objectives: To support projects enabling countries to make otherwise discarded plasma available for contract fractionation. Plasma fractionation may either be performed on a contract basis outside the country combined with re-import of the final products, or as local fractionation.
Materials and methods: Plasma quality aspects include potential virus infections (HIV, HBV, HCV) in donors to be detected by screening tests, or maximum virus amounts to be inactivated by the manufacturing process. Parameters determining the amount of viruses to be expected in the manufacturing source material (plasma pool) are the virus epidemiology in the donor population combined with the sensitivity of the screening tests.
Results: A WHO guideline was established to provide technical tools for estimating worst case scenarios of plasma pool contamination with HIV, HBV or HCV taking into account the virus epidemiology (incidence of new infections in donor subpopulations), the size of the diagnostic window (dependent on the screening test category in place, e.g. NAT, ELISAs, rapid tests) and the maximal viral load in donations not detected by the screening test applied.
Conclusions: With the establishment of the guideline by the WHO Expert Committee for Biological Standardization a tool will be available allowing estimations of maximal plasma pool contamination consistent between different countries and regions.

A24

West Nile virus – an emerging virus leading to mandatory testing in transfusion?

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Although isolated in Africa and traditionally associated as a tropical disease, West Nile virus (WNV) was occasionally found in southern and eastern parts of Europe since the 1960's. However, this did not have an implication in transfusion medicine and did not lead to additional precautions. Its natural transmission cycle involves *Culex* species mosquitoes and major hosts are domesticated and wild birds. Other modes of transmission such as solid organ transplantation and blood transfusion were described after a rapid spread across the USA beginning in 1999. Sporadic cases in Greece, Italy, Romania, and Russia were reported and some were associated with the growing trade with used tires, a well-known breeding ground for mosquitoes. Measures to enhance transfusion safety were taken rapidly. Donors living in areas with known transmission cases were excluded first, followed by nucleic acid testing (NAT) of donor sample pools. Currently, a complex system of parameters determines if and when mandatory NAT testing has been implemented. It depends on seasonality, indirectly on pathogenicity and reported cases, either human or veterinary. Amendments to the European Blood Directive should ensure a uniform process in Europe. However, general recommendations on the sensitivity and specificity of NAT are missing. Phylogenetic studies revealed that WNV can be classified into five distinct lineages. Only two major genetic lineages, lineage 1 and 2, show distinct pathogenic features; for instance, lineage 2 strains had been associated with asymptomatic infections historically but more virulent isolates were identified in Europe recently. For commercialization of NAT, specificity and sensitivity are major issues especially in times when resources are diminishing, prevalence of WNV positive donors is low, and economic requirements may have an impact on pool sizes. Changes in pool size depending on the epidemiological situation and the occurrence of neuro-invasive cases will be discussed.

Session Molecular Testing Systems for Rapid Detection of Bacteria and Antibiotic Resistance (A25 – A28)

A25

The more, the better? Multiplex PCR for the detection of gastrointestinal pathogens

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Conventional methods (culture, enzyme immunoassays, microscopy) reveal an established pathogen only in a minority of patients with infectious diarrhea. Amplification methods are not only more sensitive but also faster and thus have a higher clinical impact. However, it remains to be determined whether more targeted or highly multiplexed assays are more adequate for routine use especially in view of their high costs. Factors that may influence the selection of the appropriate test(s) include test characteristics (sensitivity, specificity), statistical facts (low prevalence of an organisms results in low positive predictive value even for tests with a high specificity), patient information (age, clinical presentation, previous antibiotics, type of institution, recent travel to a developing country), and local epidemiology.

A26

Rapid molecular detection of bacterial antibiotic resistance genes

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The increasing emergence and spread of multi drug resistant bacteria, especially *Mycobacterium tuberculosis* and *Enterobactriaceae* spp., are listed as major concerns by the world health organization (WHO). The presence of diverse ESBLs and carbapenemases in *Enterobacteriaceae* spp. and their growing prevalence has become a major challenge for health care systems in Western Europe. Moreover, carbapenemase-producing bacterial isolates pose a severe clinical problem, as non-susceptibility to beta-lactams is frequently accompanied by co-resistance to additional drug classes, e.g., aminoglycosides or quinolones. As a consequence, treatment options for carbapenemase producers are limited. Therefore, accurate and rapid identification of carbapenemase-producing *Enterobacteriaceae* is necessary for proper treatment and to limit their spread. There is a high need for diagnostic tests, which can readily be implemented in any clinical microbiology laboratory.

A27

The loss of controls – how to handle multiplex-PCR systems regarding positive and negative controls?

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New strategies for diagnosis of sepsis and meningitis include rapid multiplex PCR assays. For instance, the FilmArray (BioFire, Salt Lake City, UT, USA) system designed for improved rapid species identification in positive blood cultures serves accurate identification results of 19 bacteria, 5 yeasts, and 3 antibiotic resistance genes within one hour. Due to the new test designs, the usage of controls has changed. So far, for molecular diagnostics, the inclusion of a positive control, a negative control, and internal controls per PCR run was mandatory. The control offered by manufacturers nowadays is limited to an integrated process control replacing the internal controls. The implementation of a positive and negative control per run is impossible due to the test format; however, the majority of manufacturers recommend implementing a positive control. As a consequence, for multiplex PCR assays, multiplex positive controls as well as multiplex external quality controls should be applied in defined intervals but there are neither providers nor guidelines available so far.

A28

Molecular techniques for the diagnosis of prosthetic joint infection – is more less?

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Joint replacement is a frequently performed procedure and the number of joint arthoplasties will continue to rise. Despite low infection rates, prosthetic joint infection (PJI) is a rare but serious complication. On average, 5% of all prosthetic implants are infected by pathogens; most of them are associated with biofilms. As a consequence, germs can exhibit an increased resistance to the immune response of the host and to antibiosis. Nowadays microbiological diagnostic methods are considered as gold standard but molecular techniques are attractive tools for the diagnosis of PJI. In addition, molecular approaches are more accurate and more rapid than culture- based methods. Multiplex PCR may be used to amplify genomic DNA using multiple primers allowing direct species identification and detecting multi drug resistance. In our own study, we compared sensitivity, specificity, and clinical significance of two different commercially available microarrays to conventional culture.

Session Circulating Cell-Free DNA – Molecular Genetic Testing (A29 – A32)

A29

Cell-free circulating DNA as a new tool for screening fertility issues, fetal rhesus determination, and tumor treatment follow-up

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Real time PCR (qPCR) and high throughput sequencing (NGS) have brought significant improvements, especially through increasing the sensitivity, in the field of molecular diagnostics. However, these methodologies are also sensitive to contamination through the technician or through materials from previous sequencing runs. Fetal rhesus D determination and *EGFR* mutation detection from liquid biopsies are particularly prone to contamination. Another pitfall is the risk of delivering false negative result despite the high degree of sensitivity. To avoid such issues and to track the quality of each run, rigorous working processes have to be implemented. Measures include technician genetic profiling, strict library barcoding rules, accurate fetal versus maternal cfcDNA ratio measurement, and sensitivity and specificity validation by using external quality controls.
In conclusion, particular caution is necessary when interpreting qPCR and NGS genetic analysis results. Careful sample handling and extended validation are mandatory to avoid false positive and false negative results. Automation as well as help from dedicated expert laboratory informatics may provide improvement in terms of quality.

A30

Liquid profiling – new perspectives for prediction and monitoring of therapy response in cancer patients

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The concept of "companion diagnostics" in the treatment of cancer patients with "targeted therapies" implies the pretherapeutic therapy stratification on basis of tissue mutation status such as EGFR mutations for TKI therapies in lung cancer and absence of K-Ras mutations for cetuximab therapy in colorectal cancer. However, therapy response rates and duration are still limited due to primary or secondary resistances. Recently, genetic heterogeneity including spatial and temporal variability within a tumor or between primary tumor, lymph nodes, and distant metastases was uncovered explaining the difficulties of one-time molecular examinations in tissue biopsy material. Therefore the need for continuous monitoring of the overall mutation status in the patient body as stratification tool for therapy modification became evident.
Nowadays, sensitive blood-based diagnostics identifying mutations in circulating tumor cells (CTC) and in circulating cell-free plasma DNA (cfDNA) is available. It could overcome the genetic heterogeneity as circulating cfDNA reflects the cancerous DNA changes in the whole body. As this concept of "liquid profiling" is only minimally invasive, it can be used to complement tissue biopsy for patient stratification and for the serial monitoring of successfully treated and newly occurring resistant cell clones at an individual level. Digital PCR technologies such as

BEAMing (beads, emulsion, amplification, and magnetics) allow the sensitive quantification of specific mutations and have recently shown great potential for therapy monitoring, early recurrence detection, and resistance monitoring in colorectal and lung cancer.
In a single-center study, we demonstrated the highly sensitive detection and quantification of K-Ras mutations in plasma DNA of 32 patients with pancreatic cancer receiving chemotherapy. Most remarkably, K-Ras mutation status in plasma was highly predictive for response to chemotherapy and for prognosis of progression-free and overall survival while K-Ras tissue status was not. During the course of therapy, the amount of mutated plasma DNA correlated with therapy response and tumor recurrence. These findings underline the high clinical relevance of this "liquid profiling" approach for the individualized guidance of cancer patients.

A31

Phenotypic alterations in relation to cytogenetics in acute lymphoblastic leukemia

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Background: Acute lymphoblastic leukemia (ALL) is a disorder that mostly emerges in childhood. Approximately 80% of acute leukemias in childhood are ALL's and the majority of these cases are of B-cell origin. Phenotype determination by multicolor flow cytometry is of crucial importance.
Objectives: To investigate 39 de novo pediatric bone marrow samples from children with B-cell precursor ALL.
Materials and methods: In the majority of the cases, phenotyping was carried out by an 8-color labelling on a FacsCanto II flow cytometer for confirming the diagnosis and identifying the leukemia associated immune phenotype (LAIP). DNA index and ploidy measurements were done by propidium iodide labelling of Ficoll-Hypaque separated mononuclear nuclei fixed in 70% ethanol. Cytogenetics was carried out in parallel with fluorescence in situ hybridization (FISH) in all cases, since these techniques are the gold standards for the detection of chromosomal abnormalities and are of prognostic significance. Particular emphasis was placed on correlating a recently described novel LAIP (Kiss F. et al., Thromb. Haemost. 2006,96:176-82 and Kiss F. et al., Cytometry A 2008,73:194-201), the expression of cytoplasmic factor XIII (FXIII-A), to other laboratory variables.
Results: By flow cytometric DNA analysis, two-third of the cases were diploid (DNA index: 0.96 – 1.08), 28% were hyperdiploid (DNA index: 1.11 – 1.27), 1 case was near tetraploid (DNA index: 2.23), and another single case was hypodiploid (DNA index: 0.7). Cell cycle analysis showed that in 82% of the cases more than 90% of the pathological cells were in the G0/G1 phase. The remaining cases showed altered proliferation fractions in which a higher percentage of the cells were either in the S or in the G2+M phase. In accordance with the DNA index measurements, high hyperdiploidy (52-65) was found in 28% and frequency of t(12;21) translocation was also 28%. Normal karyotype (n=4), low hiperdiploidy (47-51) (n=3), t(9;22) (n=2), iAMP21 (n=2), near haploidy (n=1), and other aberrations (n=5) were observed in lower frequency. Overall, 59% of the cases were FXIII-A positive, 90% were CD58+ (one case had bright expression), 46% were CD66c+, 44% were CD45-, and 92% were at least partially CD34+ (>20%). Rare cytogenetic alterations predict LAIP as both cases with t(9;22) were CD66c+, but otherwise phenotype of patients with chromosomal aberrations was not significantly different from those without these alterations. Furthermore, no correlation was found between FXIII expression and recurrent chromosomal abnormalities, although FXIII negativity was found to be a poor prognostic factor in patient survival.
Conclusions: Results show that in pediatric ALL only a comprehensive analysis of surface and intracellular markers, such as nuclear TdT, cytoplasmic IgM, and FXIII-A as well as the cytogenetic analysis ensures the safety to reliably stratify patients.

A32

Implementation of v-natal® non-invasive prenatal testing for the detection of trisomies and gonosomal aneuploidies: a Swiss private laboratory experience

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Background: With non-invasive prenatal testing (NIPT) using cell-free fetal DNA (cffDNA), trisomies can be detected in the fetus, using a peripheral sample of the pregnant mother's blood. The v-natal® permits the detection of the most common chromosomal defects (trisomy 21, 18, 13 as well as maldistributions of the sex chromosomes X and Y) in the unborn child.
Objectives: To report the clinical implementation of NIPT referred to a Swiss medical diagnostic laboratory. To compare NIPT with full karyotyping.
Materials and methods: Upon on-site establishment of the verifi® technology (Illumina, San Diego, USA), validation of NIPT was performed with 126 samples. Subsequently, 753 samples obtained from pregnant women with different indications (maternal age, first-trimester screening abnormalities, IVF/ICSI, etc.) were referred for NIPT analysis (v-natal®) between July 2015 and January 2016 including the novel first trimester test regulation "risk >1 in 1000" enabling re-imbursement by Swiss statutory health insurances.

Results: In the validation stage, 8 NIPT positive samples were found (trisomies 21, 18, and 13). Results were confirmed with karyotyping. All remaining samples were confirmed to be negative. Thus, the overall sensitivity and specificity in the validation stage was 100%. Among 753 samples referred for NIPT analysis, 14 samples were classified (and confirmed externally by a second NIPT) as positive, including 9 cases with trisomy 21, 1 case with borderline trisomy 18, 2 cases with trisomy 13, and 2 cases positive for Turner syndrome (XO). Subsequent karyotyping confirmed NIPT-positive samples except of 2 NIPT results for trisomies 18 and 13, respectively.
Conclusions: NIPT improves first trimester trisomy detection after exclusion of ultrasound abnormalities. 98% of patients obtained negative NIPT reports thus avoiding further invasive procedures. Confirmation of positive results was necessary for diagnosis of chromosomal aneuploidies and to rule out placental confined mosaicism.

Poster Session (P1 – P18)

P1

Evaluation of the new cobas® HCV Genotyping Test based on real-time PCRs of three different HCV genome regions

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Background: According to the EASL recommendations on treatment of hepatitis C 2015, the hepatitis C virus (HCV) genotype and the HCV genotype 1 subtype should be determined prior to treatment initiation. Different molecular techniques may be used for determination of the HCV genotype/subgenotype including population sequencing, reverse hybridization, and primer-specific real-time PCR. The cobas® HCV GT (Roche Molecular Systems) based on primer-specific real-time PCR was brought on the market recently.
Objectives: To evaluate the performance of the new cobas® HCV GT.
Materials and methods: The accuracy of the cobas® HCV GT was determined with the Quality Control for Molecular Diagnostics (QCMD) 2014 and 2015 Hepatitis C Virus Genotyping EQA Program panels. Both panels consisted of seven plasma samples containing different genotypes of HCV and one sample negative for HCV RNA. For evaluation of the new assay, 183 routine clinical samples obtained from patients with chronic HCV infection were tested and the results were compared to those obtained by both the TRUGENE HCV 5′NC Genotpying Kit and the VERSANT HCV Genotype 2.0 Assay (LiPA). All samples showing discrepant results were additionally investigated by NS5B sequencing. Furthermore, times-to-result including hands-on-time were evaluated.
Results: When accuracy was determined, the cobas® HCV GT gave correct results for panel members containing subgenotypes 1a, 1b, and 3a. Panel members containing HCV subgenotype 5a were not detected correctly. When 183 routine clinical samples were tested, 160 results were found to be identical to those obtained by alternative assays. Seven samples gave indeterminate results with the cobas® HCV GT and results of 16 samples were found to be discrepant. 12/16 samples gave discrepant results when compared to the TRUGENE HCV 5′NC Genotpying Kit but concordant results when compared to the VERSANT HCV Genotype 2.0 Assay (LiPA) and could be confirmed with NS5B sequencing. For the remaining 4/16 samples, results obtained by the cobas® HCV GT were found to be discrepant when compared to the alternative assays and could not be confirmed with home-brew NS5B sequencing. In comparison with alternative assays, time-to-result and hands-on-time were shorter when the cobas® HCV GT was used.
Conclusions: The cobas® HCV GT showed good analytical and clinical performance. The new assay may be a good alternative for HCV geno-/subgenotyping in the routine diagnostic laboratory.

P2

Synthesis of domain III of the envelope protein of West Nile virus and its use in detection of WNV antibodies in human and horse sera

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Background: West Nile virus (WNV) is a neurotropic arbovirus of the genus *Flavivirus*. Birds are the natural host and mosquitos of the genus *Culex* usually serve as vector. Horses and humans are considered as definitive hosts. The most antigenic protein is the structural protein E of the envelope.
Objectives: To develop an immunoblot assay based on recombinant E-DIII for humans and animal sera.
Materials and methods: For standardization of the new assay, human and horse sera from Slovakia were used, which had been routinely examined by an ELISA (West Nile Compact Ingenaza, Spain). For detection of WNV-specific antibodies, a recombinant domain III of the E

protein (E-DIII) was prepared. The E-DIII encoding fragment was amplified by PCR, digested with endonucleases *Bam*HI and *Sal*I and ligated into a pQE-30 UA-mCherry-GFP plasmid, a modified pQE-30 vector UA (UA QIAexpress cloning kit, Qiagen). Electrocompetent *E. coli* DH5α was transformed with the ligated plasmid and proteins were overexpressed. The E-DIII domain was purified by nickel affinity chromatography and purity was checked by MALDI-TOF analysis. The purified E-DIII protein was blotted on a nitrocellulose membrane (Millipore, USA) and incubated with serum samples (in different dilutions). Binding of specific antibodies was detected by Protein G conjugated to horseradish, peroxidase, and chemiluminescent substrate. Signals were recorded in a Li-core scanner.
Results: Of 206 human sera, one sample was found to be positive with the new assay, which was also positive by the ELISA. Of 229 horse sera, 58 samples were found to be positive with the new assay based on recombinant E-DIII. In contrast to the ELISA, the newly-developed assay showed always clear results.
Conclusions: The recombinant E-DIII domain used in the immunoblot assay is able to detect small amounts of WNV antibodies. Furthermore, the E-DIII domain may also be used in epidemiological studies on the prevalence of WNV infection in humans and horses.
Acknowledgement: This work was supported by APVV-14-0218, VEGA 1/0261/15, and VEGA 1/0258/15. BK and PL were funded by SF project Medipark (ITMS 26220220185).

P3

Rapid in vitro protein synthesis pipeline: a promising tool for cost-effective array development for diagnostics

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Background: For the development of highly specific and sensitive serodiagnostic sets, it is necessary to synthesize and test several recombinant antigens or their truncated forms. Current protocols for protein synthesis are time consuming and require extensive laboratory work.
Objectives: To establish an alternative workflow that covers rapid construction of expression cassettes and *in vitro* protein synthesis, ready to use in the development of diagnostic tests.
Materials and methods: As a model, the C1 inhibitor and vitronectin were synthesized. With overlap extension PCR, three fragments (5' fragment consisting of T7 promoter and species-independent translation sequence; amplification products of the C1 inhibitor or vitronectin; 3' fragment encompassing GFP fusion, Myc-tag, stop codon, and T7 terminator) were fused to generate hybridized genes. The PCR generated fragment was directly mixed with the *Leishmania torentolae* lysate and incubated at 20°C for 2 hours for protein production and at 4°C overnight for protein maturation. In order to establish a cheap and user-friendly alternative to existing cell-free protein array techniques, PCR products were spotted on a hydrophobic PVDF membrane, air-dried, and covered with 1µl of *Leishmania* cell lysate (translation mix) followed by incubation as described above but in a humid chamber.
Results: Protein translation was confirmed both in-tube and on-membrane simply by screening the membrane under a laser-scanner or fluorescent microscope. The newly synthesized protein was immediately immobilized on-membrane by hydrophobic interaction with the C-terminally fused GFP.
Conclusions: The rapid protein synthesis pipeline may be used in the development of diagnostic sets (such as serodiagnostic sets), enzymatic testing of a large number of genomic expression products, and even construction of cheap protein arrays.
Acknowledgement: This work was supported by APVV-14-0218, VEGA 1/0261/15, and VEGA 1/0258/15. BK and PL were funded by SF project Medipark (ITMS 26220220185).

P4

Novel pipeline for production of a single-domain antibody – a promising tool in serodiagnostics

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Background: Single-domain antibodies (sdAbs) are novel candidates in human medicine. It has been proved that sdAbs can be used in cases where classic antibodies fail, such as disease diagnostics (serodiagnosis), inhibition of specific enzyme functions, cancer treatment, and toxin neutralization. Due to their unique properties, such as small size, well below renal clearance threshold, unique conformation, and high stability, sdAbs are perfect candidates as diagnostic tools, both *in vivo* (e.g. cancer diagnostics, HIV) and *in vitro* (e.g. ELISA tests against *Taenia solium* and *Trypanosoma spp*). Widely used methods for production of sdAbs include active immunization. Its main disadvantage is that it is time and cost consuming. Production of sdAbs based on *in vitro* stimulation of naïve B-lymphocytes by antigen of interest appears to be a suitable alternative to active immunization. However, this method has never been used for production of sdAbs.

Objectives: To establish a new pipeline for *in vitro* induction of B-lymphocytes and production of antigen-specific sdAbs against OspA of borrelia.
Materials and methods: A DNA fragment encoding OspA of SKT-7.1 was amplified, cloned in the pQE-30 UA vector, and over-expressed in *E. coli*. OspA was then used as antigen in *in vitro* immunization of B-cells isolated from peripheral blood of healthy *Llama glama*. Following mRNA isolation and cDNA synthesis, the variable fragment of heavy-chain antibodies was cloned and displayed on the surface of an M13K07 phage. After one round of biopanning with immobilized OspA, seven phage clones expressing sdAbs on their surface were used in a phage dot blot to confirm their affinity to recombinant OspA. As a negative control, the identical procedure was performed to select sdAbs from B-lymphocytes that were not subjected to stimulation with OspA.
Results: All phage clones chosen showed positive interaction with OspA. With the immunized library, a hundred-fold higher yield of positive clones was achieved. This improvement is important for selection of the most suitable sdAb.
Conclusions: The method described is convenient for production of sdAbs suitable for subsequent applications in disease diagnosis and treatment.
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P5

EGFR mutation analysis in non-small cell lung cancer: evaluation of a novel sensitive strip-based reverse hybridization assay

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Background: Lung cancer is one of the most common human cancers and a leading cause of mortality worldwide. In patients with non-small cell lung cancer (NSCLC) epidermal growths factor receptor (*EGFR*) mutations have been associated with tumor response to treatment with targeted *EGFR* tyrosin kinase inhibitors. Sanger sequencing with limited analytical sensitivity (e.g. 20%) is widely used for mutation detection.
Objectives: To evaluate the potential of a novel strip-based reverse hybridization assay to sensitively detect *EGFR* mutation in DNA from NSCLC tissue samples.
Materials and methods: Formalin-fixed paraffin-embedded (FFPE) DNA samples from 59 patients with histologically confirmed primary NSCLC tumor were used to compare *EGFR* mutation analysis between the *EGFR* StripAssay® (ViennaLab Diagnostics GmbH, Vienna, Austria) and Sanger sequencing. An appropriate paraffin block containing tumor tissue was selected for analysis after reviewing the hematoxilin-eosin (HE) stained slides. An area of tumor on the HE stained slide was marked and micro-dissected on a corresponding unstained slide so that samples contained at least >50% tumor cells. The strip-based reverse hybridization assay covered 16 mutations in exons 18-21 of the *EGFR* gene. For Sanger sequencing primer pairs for *EGFR* exons 18-21 were used. Data were aligned to the reference sequence NM_005228.3 using SeqScape Software (Life Technologies, Vienna, Austria).
Results: The strip assay identified 7 (11.8%) of 59 FFPE samples to carry an *EGFR* mutation of which 4 (57.1%) and 3 (42.8%) samples were positive for exon 19 and 21 mutations, respectively. Of note, no sample was identified with *EGFR* exon 18 or 20 mutations. All mutations found with reverse hybridization assay (strip assay) were confirmed by Sanger sequencing with a concordance of 100%. The analytical sensitivity of the strip assay was 1%.
Conclusions: The strip assay presented here is an accurate and sensitive diagnostic tool for the detection of *EGFR* mutations in genomic DNA isolated from FFPE tissue. This method could be considered as an alternative protocol to Sanger sequencing for *EGFR* mutation analysis on limited quantity samples.

P6

Coronary risk and genetic markers

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Background: Coronary artery disease is the leading cause of death worldwide. Accurate risk prediction is important to decide if medication is warranted and which type and dose of a drug is appropriate. To estimate a person's risk several algorithms have been developed including the Framingham Calculator (Risk Assessment Tool for Estimating Your 10-year Risk of Having a Heart Attack). This algorithm is based on personal and clinical data and has proven useful for prediction of coronary risk. However, the underlying scoring system does not take individual

genetic data into account. Recently, genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with coronary artery disease. Interestingly, the majority of them is neither located within a gene, nor near a gene known to be associated with coronary risk.
Objectives: To improve coronary risk prediction by adjusting the Framingham risk calculation for the presence/absence of individual risk alleles.
Materials and methods: Eight non-redundant SNPs were chosen from GWAS and included into the Framingham risk calculation. These SPNs show a highly significant association with coronary risk ($p<10^{-6}$ or less) and are not associated with established risk factors.
Results: Correction of conventional Framingham calculation for individual patterns of risk alleles can affect risk estimates considerably. For instance, the conventional risk of a 53 year old male may vary between 5.5 and >30% depending on his personal risk allele pattern.
Conclusions: We were able to show that inclusion of individual SNP profiles into the Framingham risk calculation may have a considerable impact on risk prediction. This could lead to changes in the therapeutic regimen and may be of particular importance for individuals who would otherwise be classified as low or medium risk but, in fact, are at high risk of coronary artery disease due to their genetic makeup.

P7

Osteoporotic fracture risk and genetic markers

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Background: According to the WHO, osteoporosis belongs to the 10 most important diseases worldwide, affecting over 200 million people. In Central Europe, approximately 10 % of the population is affected. The main complication are osteoporotic fractures causing lifelong need of care in over 50% of patients and leading to death in about 30% of individuals over 70 years of age. Measurement of bone mineral density with dual energy X-ray absorptiometry (DXA, DEXA) is widely used to predict fracture risk. However, this method only detects about 25% of people who will actually suffer an osteoporotic fracture. Another established method is the use of an algorithm to predict the 10-year fracture risk based on personal and clinical data, e.g. the FRAX calculator of the WHO. Recently, genome-wide association studies (GWAS) have identified over 50 single nucleotide polymorphisms (SNPs) associated with bone mineral density[2)]. Some of them are particularly associated with bone fracture ($P < 5 \times 10^{-4}$). For each risk allele the frequency in the population and the impact on fracture risk (odds ratio) are known; however, the FRAX calculator does not take into account individual genetic data.
Objectives: To improve FRAX risk prediction by adjusting for the presence/absence of individual risk alleles.
Materials and methods: Fourteen SNPs from GWAS associated with increased osteoporotic fracture were chosen and incorporated into the risk calculation. These SNPs have been identified in Caucasians and were replicated in various studies.
Results: Correction of conventional FRAX calculation for different patterns of these risk alleles can affect the risk estimate considerably. For instance, a 60 year old female with a conventional risk of 14.0% may, in fact, have a risk between 6.7 and 29.6% depending on her personal risk allele pattern.
Conclusions: We were able to show that FRAX risk prediction can be considerably modified when individual SNP patterns are taken into consideration. This could be of particular clinical and therapeutic importance for individuals who would otherwise be identified as being at low or medium risk, but are in fact at high risk due to their genetic makeup.

P8

Detection of respiratory viruses in clinical samples

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Background: Acute respiratory tract infections are among the commonest causes of morbidity and mortality, particularly in children and in immunocompromised patients. They also account for numerous hospitalizations every year. Polymerase chain reaction (PCR) is the preferred diagnostic method for the detection of respiratory viruses. Simultaneous infection with more than one pathogen is common and the detection of a multiple viral and bacterial infection in one single test is only feasible using multiplex PCR. A reliable multiplex PCR assay is essential for fast diagnostics.
Objectives: To compare two different multiplex real time assays for the detection of respiratory viruses.
Materials and methods: The identification panels included RNA viruses, DNA viruses, and bacteria. In total, 104 specimens of hospitalized patients showing respiratory symptoms were tested using Argene r-gene® Multiplex PCR (MWS) (bioMerieux) kits and an in house multiplex qPCR. Nucleic acid (NA) extraction for all samples was done using the NucliSENS® easyMag® (bioMerieux) platform. When the MWS kits were

employed, PCR mixes were processed with the easySTREAM™ (bioMerieux) liquid handling system (automated pipetting platform), whereas the in house PCR mixes were pipetted manually.
Results: Of 104 clinical specimens, 91 (87.5%) were positive. With MWS kits for detection of adenovirus, bocavirus and parainfluenza virus, and rhino-/enterovirus, 5% more positives were detected than with the in house assay. For respiratory syncytial virus (RSV), more samples tested positive with the in house PCR. However, 10 of 14 RSV-negatives obtained with the MWS kit were showed a low positive result (Ct >30) with the in house assay.
Conclusions: The combination NucliSENS® easyMag®, easySTREAM™, and MWS kits is a fast and easy-to-use method for the detection of respiratory pathogens. Automatic pipetting is reliable and reduces the hands-on time. MWS r-gene® Multiplex PCR kits show a super performance compared with in house PCR for detection of adenoviruses, bocaviruses, parainfluenza viruses, and rhino-/enteroviruses, while a higher number of RSV was detected by the in house assay.

P9

Bioinformatics analysis of B-cell epitopes of surface proteins of *Neisseria (N.) meningitidis* for downstream development of immunodiagnostics

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Background: Gold standard serological diagnostic methods focus on antigens and their epitopes. The use of bioinformatics to identify linear and conformational B-cell epitopes of *N. meningitidis* is a new approach. *N. meningitidis* is serologically classified based on structural differences of its capsule (serogroup), major outer membrane porin proteins (serotype), and other outer membrane proteins (serosubtype). Three surface-exposed antigenic proteins, PilE, OpcA, and NadA may be subjected to B-cell epitope prediction.
Objectives: To predict linear and conformational B-cell epitopes of surface exposed proteins, which may be used to develop highly specific and sensitive serodiagnostic tests and even an epitope-based vaccine.
Materials and methods: Primary amino acid sequences of three proteins (PilE, OpcA, and NadA) were retrieved from the RCSB Protein data bank and subjected for consensus technique of epitope prediction. ABCpred and LBtope were used to predict linear epitopes, while COBEpro was applied for predicting discontinued epitopes. ElliPro and Epitopia were applied to predict both types. For visualization of the predicted epitopes, Geneious pro 9.0 was applied.
Results: The combination of algorithms to predict linear epitopes generated 7 common linear epitopes on the PilE, 11 on the OpcA and 7 on the NadA proteins using a threshold score >0.7. All in all, 2 conformational epitopes were predicted on PilE (score >0.7), OpcA (score >0.7), and NadA proteins (score >0.6).
Conclusions: This study provides the proof-of-principle evidence of the feasibility to use bioinformatics for identification of novel targets for immunodiagnostics employing proteins that are highly antigenic and/or take part in the invasion process.
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P10

Evaluation of combined *SFRP2* promotor methylation and *KRAS* mutation analysis for colorectal cancer screening in fecal DNA

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Background: Colorectal cancer (CRC) is a major cause of cancer death worldwide. No fully international consensus exists on stool-based genetic and epigenetic colorectal cancer (CRC) screening markers.
Objectives: To assess the potential of combining secreted frizzled-related protein 2 (*SFRP2*) promotor methylation analysis and the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation status to improve CRC detection rates in fecal DNA.

Materials and methods: SFRP2 promoter methylation was investigated in stool DNA isolated from 18 CRC patients and 22 healthy controls using a prototype non-quantitative reverse-hybridization (RH) assay based on methylation-specific DNA amplification followed by RH of biotinylated amplicons to sequence-specific methylation detection probes with MethyLight serving as a reference method. *KRAS* mutational analysis was performed using a commercially available RH assay (*KRAS* StripAssay; ViennaLab Diagnostics GmbH, Vienna, Austria) designed for the sensitive detection of 10 mutations in codons 12 and 13.
Results: *SFRP2* promotor methylation and *KRAS* mutation status as single markers showed a sensitivity and specificity for CRC detection of 77.7% and 77.3%, and 27.7% and 77.3%, respectively. *SFRP2* promotor methylation analysis by RH showed a lower sensitivity (61.1%) but a higher specificity (86.3%) compared to MethyLight (sensitivity: 77.7%; specificity: 77.3%). The combination of *SFRP2* promotor methylation as determined by MethyLight and *KRAS* mutational analysis demonstrated a sensitivity and specificity of 83.3% and 54.5%, respectively. Statistically significant differences in fecal DNA methylation for *SFRP2* were found between CRC patients and healthy controls ($p < 0.001$).
Conclusions: Combined analysis of *SFRP2* promotor methylation as determined by MethyLight and *KRAS* mutation status resulted in a slight increase in sensitivity but a distinct decrease in specificity compared to *SFRP2* promotor methylation as single marker. Hence, our findings, although limited in size, do not support the use of combined *SFRP2* methylation and *KRAS* mutation testing for CRC screening in stool.
However, further prospective studies are needed to optimize and standardize the clinical utility and widespread introduction of fecal DNA markers for CRC detection in clinical routine.

P11

Evaluation of a *Klebsiella oxytoca* toxin PCR for diagnosis of antibiotic-associated hemorrhagic colitis in fecal specimens

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Background: Toxin-producing *Klebsiella* (*K.*) *oxytoca* causes antibiotic-associated hemorrhagic colitis (AAHC). This special form of antibiotic-associated colitis presents with bloody diarrhea and segmental colitis mainly during a therapy with beta-lactam, especially penicillins. Recently, the responsible cytotoxin tilivalline produced by certain *K. oxytoca* isolates was identified, together with genes essential for its production including nonribosomal peptide synthetase genes A and B (*nspA* and *nspB*).
Objectives: To establish and evaluate a PCR assay for diagnosis of toxin-positive *K. oxytoca* causing AAHC in fecal specimens.
Materials and methods: A LightMix® (TibMolbiol) was designed including targets for *K. oxytoca*, *nspA*, *nspB*, and phocine herpesvirus (PhHV) as internal control. DNA was extracted on the NucliSENS® easyMAG® (bioMerieux) instrument. Amplification was done on the LightCycler® 480II (Roche) instrument. Analytical sensitivity was determined with a dilution series from $1.5x10^6$ to 1.5 CFU/ml using the AHC-6 clinical isolate. For analytical specificity, 86 bacterial species were tested. To determine the clinical sensitivity and specificity, residual fecal specimens with specified request for *K. oxytoca* were tested. Toxigenic culture served as reference method.
Results: The analytical sensitivity was found to be 15 CFU/ml for all targets. Analytical specificity for *K. oxytoca* was 100% and for both toxin genes 95%, respectively. So far, 53 clinical fecal specimens were tested. With culture, 11 (20%) *K. oxytoca* isolates were detected; of these isolates, 8 were identified as "toxigenic strains". With PCR, 17 (32%) *K. oxytoca* positives were identified; of these isolates, 13 were toxin positive. The PCR assay showed a sensitivity of 100% (95% CI of 48% to 100%) and a specificity of 83% (95% CI of 70% to 93%) with a positive predictive value of 38.5% (95% CI of 14% to 68%) and a negative predictive value of 100% (95% CI of 91% to 100%).
Conclusions: Currently, no commercial molecular system is available for detection of tilivalline-positive *K. oxytoca* in fecal specimens obtained from patients with symptoms suspicious of AAHC. The established PCR assay appears to be a highly sensitive method to improve diagnosis of *K. oxytoca* causing AAHC.

P12

Dried blood spots as sample material for HCV viral load monitoring using the COBAS® AmpliPrep/COBAS® TaqMan® HCV Test v2.0 – a simple and effective method

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Background: With the availability of highly efficacious all-oral drug combinations for the therapy of chronic hepatitis C (HCV), treatment can now expand into areas where an advanced laboratory infrastructure may not be available. In such areas, the use of dried blood spots (DBS) is a cheap and simple solution to ship patient samples from the collection site to a reference laboratory.

Materials and methods: Samples from 48 chronically HCV infected patients were used to generate DBS using Whatman 903TM Protein Saver Cards with five spots each. Spots were prepared with 35 µL of whole blood and left to dry overnight at room temperature before storage in individual zip bags containing one desiccant bag. Each individual DBS was folded in half with the blood spot facing inwards and inserted into a COBAS® AmpliPrep (S-tube). Two procedures were compared for the DBS testing: Specimen Pre-Extraction Reagent (SPEX) with heating (SH) and SPEX without heating (SWO). For both procedures, 675 µl of Roche SPEX were added to each S-tube. Three replicates of the same specimen were tested for each procedure. For the SH procedure, S-tubes were incubated for 10 min at 56°C (shaking at 1000 rpm) on a thermomixer. After incubation, each S-tube was loaded onto the COBAS® AmpliPrep/COBAS® Taqman® (TaqMan®) instrument for nucleic acid extraction, amplification, and detection according to the instructions for use of the COBAS® AmpliPrep/COBAS® Taqman® HCV Quantitative Test v2.0 (TaqMan® HCV v2). For the SWO procedure, each S-tube was incubated for 60 min at room temperature. After incubation, S-tubes were loaded onto the system as for SH. Mean (n=3) DBS viral load (VL) results were compared to correspondent plasma VL.
Results: All testing was performed using TaqMan® HCV v2. Plasma viral loads varied from 3.5 to 7.7 $\log_{10}$ IU/ml. Correlation between DBS and plasma was linear for both procedures: y = -0.0176x-1.4262 (R^2= 0.97) and y = -0.0369x-1.395 (R^2= 0.96) for SH and SWO. Mean titer difference to plasma was -2.00 log IU/ml for SH and -1.97 log IU/ml for SWO. After correction for volume influences, the mean titer differences to plasma significantly improved to -0.39 and -0.36.
Conclusions: HCV VL measurements with TaqMan® HCV v2 using DBS correlates to plasma and may be performed even without a need of a heat based extraction step. HCV VL monitoring using DBS appears to be simple alternative compared to plasma in remote settings.

P13

Evaluation of the Quidel AnDiaTec® CMV real time PCR Kit with various patient sample matrices

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Background: Lower Austria's largest hospital laboratory is located at the University Clinic St. Poelten. Since 2010, the laboratory is ISO certified and around 40% of all laboratory tests performed in Lower Austria's hospitals are provided. Highest diagnostic standards are maintained and thus all newly available products are compared to assays currently established for routine diagnostics. Quantitative cytomegalovirus (CMV) real time PCR analysis is one of the high throughput testing parameters and a correct CMV DNA result is of utmost importance.
Objectives: To evaluate the new commercially available Quidel AnDiaTec® CMV real time PCR Kit and to compare it with the bioMerieux CMV R-gene® kit. Both kits have been IVD/CE-marked.
Materials and methods: Serum, plasma, EDTA whole blood, and urine samples from 8 patients with previously positive CMV test results were used. Samples were tested undiluted and in 3 serial dilutions down to the detection limit of the two assays in order to compare their limits of detection. A total of 128 samples were generated and all samples were extracted using the automated MagnaPure LC 2.0 system (Roche Diagnostics). After extraction, eluates were directly used for real time PCR analysis in both assays on a LightCycler 480 II instrument (Roche Diagnostics).
Results: Three of eight urine samples were found to be negative in both assays and excluded from calculation. All other samples were positive with at least one of the real time PCR assays. With the R-gene® kit, 72 positive and 44 negative results were obtained. The AnDiaTec® kit gave 85 positive and 31 negative results. The R-gene® kit detected 8 positives that were negative with the AnDiaTec® kit, whereas this kit detected 21 positives that were negative in the R-gene® kit.
Conclusions: The AnDiaTec® kit performed better than the R-gene® kit (P=0.068). This new product for quantitative CMV testing can thus be recommended for use in a routine diagnostic laboratory.

P14

Mutations in the core region of hepatitis C virus in patients with chronic hepatitis C are associated with advanced liver disease

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Background: Hepatitis C virus (HCV) is a globally prevalent pathogen with diverse clinical outcome. HCV core protein is complex, capable of modifying hepatitis C virus life cycle by participating in interactions with host proteins, altering cellular signaling pathways. HCV core gene mutations and subsequent amino acid changes were reported to be associated with advanced liver disease and increased risk of hepatocellular carcinoma.
Objectives: To investigate the mutations in HCV core gene and amino acid substitutions in 87 patients with chronic hepatitis C. To correlate mutations with clinical and pathohistological parameters (hepatitis activity, grade, and fibrosis) of liver disease.

Materials and methods: HCV RNA was isolated from serum samples of 85 HCV infected, therapy naïve patients collected at first diagnosis at Clinical Hospital Center Rijeka, Croatia. 28 (32.9%) patients were infected with HCV subgenotype 1a, 30 (35.3%) with HCV subgenotype 1b, and 27 (31.8%) with genotype 3a. Viral RNA was transcribed to cDNA and the HCV core gene was amplified by a nested PCR protocol using sets of primers covering the entire core region. Amplification products were sequenced with the Big Dye Termination v1.1 method on an ABI 310 sequencer. HCV core sequences were compared with referent HCV genotypes from Gene Bank.
Results: This study revealed that the most frequent and statistically significant mutated amino acids were R70Q/H, T75A, and M91L/C (p=2.8 x 10^{-5}, p=1.9 x 10^{-12}, and p=1.7 x 10^{-5}, respectively), present in HCV subgenotype 1b. Presence of R70Q/H, T75A, and M91L/C mutations were significantly correlated with HCV subgenotype 1b and basal viremia. Pathohistological parameters were associated with presence of R70Q and M91L/C mutations. In addition, all samples including subgenotypes 1a, 1b, and 3a showed 10 to 20 sense mutations indicating a high mutation rate in the core region of HCV.
Conclusions: Specific amino acid substitutions in the HCV core region appear to be correlated with progression of liver disease and may serve as prognostic marker for progression of liver disease.

P15

B-cell mimotope mapping of anti-*Borrelia bavariensis* antibodies by a phage display peptide library

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Background: Lyme disease is a tick-borne infection caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex. The complexity and differential expression of borrelial antigens has posed challenges for the serodiagnosis of borreliosis. It is generally believed that use of truncated forms of highly immunogenic borrelial proteins may remove the cross-reactive epitopes and is likely to increase both the sensitivity and specificity of immunoassays.
Objectives: To identify mimotopes of borrelial antigens and include selected peptides interacting with polyclonal serum antibodies to a chimeric molecule with diagnostic potential.
Materials and methods: Immunodominant proteins OspA, OspB, and BmpA of the neuroinvasive *Borrelia bavariensis* were overexpressed in *E. coli* used for isolation of specific polyclonal antibodies from human sera with nickel-charged affinity resin. Solution-phase biopanning of the combinatorial peptide phage library Ph.D.-C7C was performed using Protein G/A-magnetic beads. Each immunoreactive mimotope was sequenced and screened *in silico* with the help of web-available tools (SAROTUP 2.0). The peptides with potential to mimic natural antigenic epitopes recognized by serum-specific antibodies were tested using phage a dot blot.
Results: Novel oligopeptides were identified by phage display library screening. Eight 7mer mimotopic sequences were selected according to bioinformatics analysis and phage dot blot; 4 for OspA (CSWNQMRGC, CRSTLQHSC, CSSMRMDQC, and CKAAMVSSC), 2 for OspB (CGGG-PLYMC and CDTHKYGIC), and 2 for BmpA (CMAPDSRVC and CIHSVKTEC).
Conclusions: Identified mimotopes in the chimeric construct may be used to develop a more specific diagnostic set for neuroborreliosis.
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P16

Rapid detection of new combinations of carbapemenase-genes using Genspeed® Superbug CR: a useful tool to eliminate their spread

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Background: Carbapenemase-producing bacteria constitute a dramatic problem worldwide. Most of the carbapenemase-encoding genes are located on transferable genetic elements that are often associated with other antibiotic resistance genes, leading to their rapid transfer and facilitating the spread of uncontrollable superbugs.
Objectives: Aim of the present study was to evaluate the Genspeed® Superbug CR assay to detect new combinations of carbapenemase-genes directly in clinical samples.
Materials and methods: A total of 245 surveillance fecal specimens (68 stool samples and 177 rectal swabs) were taken from patients, all of them hospitalized in various wards of the University Hospital of Larissa, Central Greece. The specimens were tested by the molecular assay

Genspeed® Superbug CR (Greiner Bio-One, Kremsmünster, Austria) that is able to detect the genes bla_{VIM}, bla_{KPC}, bla_{NDM}, and bla_{OXA-48} directly in the clinical specimen. Confirmation of results was done after cultivation of samples on MacConkey agar supplemented with 1 mg/L imipenem. All isolates recovered from culture were identified by the automated VITEK 2 (BioMerieux) system, while susceptibility testing was done by VITEK 2 and by Etest. The detection of the genes encoding carbapenemases was assessed by PCR; molecular characterization was performed by multilocus sequence typing.
Results: Of 245 specimanes, 94 (38.4%) were found to carry carbapenemase genes. Sixty eight specimens were found to be positive for only one carbapenemase gene (25 were positive for bla_{KPC}, 19 for bla_{VIM}, 22 for bla_{NDM}, and 2 for bla_{OXA-48}), while 26 isolates, all *Klebsiella pneumoniae*, were positive for more than one gene. In more detail, 16 were positive for bla_{VIM} and bla_{KPC} genes, 5 were positive for bla_{VIM} and bla_{NDM} genes, and 5 were positive for bla_{KPC} and bla_{NDM} genes. To our knowledge, the two combinations bla_{VIM} / bla_{NDM} and bla_{KPC} / bla_{NDM} are reported for the first time.
Conclusions: Genspeed® Superbug CR, having the ability to detect new combinations of resistance genes directly in clinical samples, is a useful tool for rapid diagnostics and simple surveillance procedures. The results, available within two hours, were in very good agreement with culture and PCR.

P17

Near patient infectious disease testing using point of care technology with the cobas®Liat system

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Background: A paradigm shift in near patient assessment with point of care molecular diagnostic testing on the cobas® Liat System provides an opportunity to bring sensitive and specific PCR technology to the bedside, pharmacies, long term care facilities, and physician offices. The cobas® Liat Influenza A/B Assay is a rapid, automated diagnostic tests for qualitative detection of influenza virus. The assay is performed on the cobas® Liat System, which automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples.
Objectives: To evaluate the workflow and ease of use of the cobas® Liat Influenza A/B Assay.
Materials and methods: The cobas® Liat platform is a true point of care instrument which is comprised of two principal components: The pencil sized flexible tube (cobas® Liat Assay Tube) which accepts clinical material and contains all necessary components required for sample extraction, amplification, and detection. The cobas® Liat Analyzer automates reagent preparation, target enrichment, inhibitor removal, nucleic acid extraction, amplification, and detection through sample processors containing temperature control elements which manipulate the reagents within the cobas® Liat Assay Tube. Five laboratories in four European countries tested a blinded panel of five nasopharyngeal specimens. Each panel contained two Influenza negative samples, two Influenza B positive samples and one Influenza A sample and was comprised of unique specimens. The samples were transferred into the Liat tubes using the pipette provided with the tube. No pre-analytic steps were required.
Results: The cobas® Liat Influenza A/B Assay provides patient results in 20 minutes. The five panels executed by six different operators were all concordant with the reference method (D3 Ultra™ DFA Respiratory Virus Screening & ID Kit).
Conclusions: The cobas® Liat Influenza A/B Assay is a simple to use molecular diagnostic platform that provides fast results. The system offers true point of care patient management and quick turn-around time for low throughput laboratory testing.

P18

Fully automated platform for the routine determination of immunosuppressant drugs in whole blood

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Background: Therapeutic drug monitoring of immunosuppressant agents needs to be accomplished by extremely accurate techniques. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) shows higher sensitivity and superior specificity compared to

immunoassay-based approaches; however, LC-MS/MS approaches lack standardization. To increase the data quality, safety, and throughput of LC-MS/MS quantitation of immunosuppressant drugs, a fully automated platform for the quantitation, the CLAM-2000 (Shimadzu), has been introduced.

Objectives: To determine levels of major immunosuppressant drugs using the novel fully automated CLAM-2000 preparation unit and to compare results obtained with an immunoassay.

Materials and methods: The analysis of immunosuppressants was performed using the automatic preparation unit CLAM-2000 online with HPLC-MS (NexeraX2-LCMS-8050, Shimadzu). Blood samples (EDTA whole blood) were collected in standard blood collection tubes and loaded directly into the preparation unit. Reagents (extraction buffer, precipitation reagent), reference samples, calibrators, and internal standards from the MassTox® kit (Chromsystems 93000) were reconstituted and loaded into the preparation unit. The fully automatic preparation/analysis procedure steps were: a) Transfer of 25 µl of blood sample from the collection tube to a disposable filtration-collection vial; b) addition of 50 µl of extraction buffer (liquid-liquid extraction); c) addition of 12.5 µl of internal standards mix; d) stirring and incubation for 2 min; e) addition of 125 µl of precipitation reagent; f) stirring and filtration for 2 min (deproteinization); g) transfer of the disposable vial containing the filtrated sample into the LC autosampler for injection (5 µl). LCMS-MS quantification was performed following the analytical conditions suggested by the MassTox® kit.

Results: When the accuracy of the new test system was determined by using reference material, results were obtained close to those expected (bias <5%). The LOQ for the 4 drugs tested was found to be 0.5 µg/l. A good linearity over a $6\log_{10}$ range was observed (r^2=0.997). Determination of the intra-day imprecision with the automated sample preparation resulted in CVs between 2.5 to 8% for the compounds tested. The inter-day imprecision was found to be in accordance with CLSI guidelines, with CVs of less than 12%. In comparison to an immunoassay-based assay, results obtained with the new test system showed a good correlation.

Conclusions: The completely automated quantification method for Immunosuppressant drugs allows routine analysis with high data quality, reduced time, and increased throughput.

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