 DOI 10.1515/cclm-2018-0281 — Clin Chem Lab Med 2018; 56(6): eA97–eA120

Abstracts*)

ISMD2018
Twelfth International Symposium on Molecular Diagnostics

Medical University of Graz,
Austrian Society for Laboratory Medicine and Clinical Chemistry

Graz, May 31 – June 02, 2018

Organizers

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Plenary Session – 20th Anniversary of ISMD (A1, A2)

A1

Molecular Diagnostics from the cradle to the grave

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In the last decade, scientific advances have laid a solid foundation for the development of routine molecular 'liquid biopsies'. Cell-free DNA and RNA biomarkers can complement or even surrogate tissue biopsy and have been introduced in clinical laboratory practice, especially in prenatal screening and oncology.
Through cell-free DNA detection by real-time PCR techniques and next generation sequencing, a simple non-invasive test becomes a liquid biopsy for 1) aneuploidy testing in the context of first-trimester screening or 2) cancer, surveying a patient's circulation with the goal of early detection, information on prognosis, personalized therapies, and monitoring for recurrence or resistance.
The novel Swiss reimbursement scheme for trisomy 21 and 13/18 NIPT testing in patients with calculated first-trimester risks of approximately 1:10 – 1:1000 implemented in 2015 has led to a 2/3 reduction in invasive procedures. For IVF/ICSI patients, "day 5 NIPT" examinations are now possible by whole genome analysis from trophectoderm biopsies prior to selective embryo transfer.
Targeted gene panels tested on tissue biopsies are "gold standard" in the context of somatic mutation-based targeted tumor therapy. However, already in single patients where surgery is not possible "liquid biopsies" are requested for resistance mutation detection in the context of second line therapies.
Molecular 'liquid biopsies' have become a central piece in the clinical laboratory and surgical pathology. Further developments and test formats are needed for early detection of cancers in combination with protein biomarkers to achieve a less invasive and more precise personalized medicine.

A2

Is preterm birth an infectious disease? Implications for risk identification and prevention

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Background: Birth before 37 weeks gestation is increasing in the developed world and remains the major cause of perinatal mortality. Of the children who survive extreme prematurity (less than 26 weeks), 22% will develop severe disability. While the etiology of spontaneous preterm labor remains elusive, a role for bacteria and the associated maternal inflammatory immune response is now recognized as a potential trigger in some cases.
Objectives: To establish if spontaneous preterm birth of infectious etiology stems from a combination of the presence of certain components of the vaginal microbiota and a failure of maternal immune defenses to prevent bacteria from ascending the cervix and colonizing the membranes and placenta.
Materials and methods: Samples from women at risk of preterm birth were recruited in the UK and Malawi. Flow cytometry on mid-trimester cervical cytology brushes was used to identify and quantitate leucocytes. Next generation sequencing and specific quantitative PCRs were used to quantitate and establish vaginal and placental microbiota from women delivering at term and preterm.
Results: Severe chorioamnionitis was associated with distinct differences in bacterial community members, a higher bacterial load and lower species richness. The placental microbiota was found to differ between very preterm and term deliveries. The absence of macrophages identified women at a 3-fold increased risk of giving birth before 34 completed weeks of gestation. Absence or low levels of other mucosal immune factors in the preterm group added further specificity to risk prediction. We also found that specific bacteria found concurrently on placental tissues associate with chorioamnionitis and delivery of smaller newborns. The vaginal microbiota was also found to be a potential additional risk factor for preterm birth.
Conclusions: These studies provide evidence for the role of bacteria in preterm birth. Bacterial DNA is present in the majority of placental membranes from both term and preterm deliveries, irrespective of mode of delivery and there are consistently identifiable distinct bacterial species found in preterm labor. The relative roles of bacterial diversity, bacterial load and host in the induction of preterm birth are under further investigation. The presence of phagocytic leukocytes at the distal cervix and endocervical canal, and the chemokines that support their migration are features of pregnancies that are likely to go to term, and failure to detect even a small number of leukocytes by highly sensitive flow cytometric methods is a marker of heightened risk of preterm birth. A greater understanding of how bacteria are related to preterm delivery should lead to novel ways of limiting preterm births.

New Technologies (A3 – A12)

A3

HIV and HCV genotyping and drug resistance monitoring using targeted Ion Torrent NGS: an update

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Background: While HIV drug resistance monitoring (DRM) has become a mandatory component of HIV drug therapy, HCV genotyping and detection of resistance associated substitutions (RASs) also remain relevant decision making tools even in view of highly efficient drug treatment with 2nd generation DAAs. Respective laboratory methods such as Sanger sequencing and line probing have come of age and in many instances are gradually replaced by next generation sequencing technology.
Objectives: To develop and clinically evaluate NGS workflows for drug resistance detection and genotyping in patients with HIV or HCV infection.
Materials and methods: Automated workflows have been developed using robotic sample preparation followed by Ion Torrent NGS library preparation and sequencing in conjunction with bioinformatics interpretation software generating both comprehensive quality and clinical reports readily usable by the operator. Therapy relevant mutations are detected in HIV RT, protease and integrase genes, while NS3, NS5A, and NS5B are target regions for HCV. Genotyping is based on HCV NS5B sequencing.
Results: Using this workflow, several international studies conducted in Europe, the US, and Asia, respectively, have shown highly accurate detection of HIV DRMs and HCV RASs as well as superior genotyping. In a blinded comparison study by Stanford University, 99% concordance was demonstrated for major mutations in the RT and protease genes. French, German, and Asian reference centers confirmed the reliable performance of the test system.
Conclusions: The availability of regulatory approved automated NGS for drug resistance monitoring (HIV and HCV) and highly accurate genotyping (HCV) is changing the testing landscape. Its prominent features are comprehensive mutation coverage, highly increased accuracy and sensitivity, short turn-around time, and cost savings.

A4

Update on the RoboGene® Kit for HDV RNA Quantification (CE-IVD)

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Background: Hepatitis D represents a major and life-threatening health burden in certain areas of the world. Confirmation of hepatitis infection as well as monitoring viral load is urgently needed in order to verify patients' therapeutically requirements and forward the development of new, specifically targeted anti-HDV therapies. The first CE-IVD labeled one-step real-time PCR kit for quantification of HDV RNA, the RoboGene® HDV RNA Quantification Kit 2.0 (Analytik Jena), represents a fully adequate tool for improved management of HDV infection. Since acquiring CE-IVD status in 2015, the assay has been further evaluated and is now available in an improved kit format in order to provide the perfect solution from low to higher throughput laboratories including adaption to automated workflows.
Objectives: To confirm the diagnostic validity of the RoboGene® Kit implementing different evaluation studies.
Materials and methods: At the MVZ Labor Volkmann, a total of 109 HDV RNA-positive patient samples were analyzed and quantitative data were compared with results previously obtained with an in-house assay. At the Hannover Medical School, the RoboGene® Kit was compared with an in-house assay in a longitudinal study: 15 HDV IgG-positive patient samples were tested before as well as 12 and 48 weeks after treatment with pegylated interferon-alpha. At the French National Reference Laboratory, HDV RNA-positive samples including all 8 known HDV genotypes were tested: 1:10 dilutions were prepared and results were compared to those obtained by the routinely used assay.
Results: For 109 HDV RNA-positive samples, results were comparable between the two assays as shown by linear regression ($R^2 = 0.85$). Scattered negative samples were correctly recognized as HDV RNA-negative. In the longitudinal study, the RoboGene® Kit resulted in a very low risk for false-negative results. The kit was also able to detect all different HDV genotypes. Compared with the routinely used assay, the RoboGene® Kit resulted in lower values of the HDV load but performed well on African genotypes usually being most challenging for the majority of assays available.
Conclusions: With its outstanding sensitivity, the RoboGene® Kit is especially useful for evaluation of response to anti-HDV therapy independently of the underlying genotype.

A5

Evaluation of the Siemens VERSANT® kPCR molecular system with Fast Track Diagnostics respiratory multiplex polymerase chain reaction respiratory panels

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Background: Respiratory tract infections are the leading cause of patient visits to the physician. Common colds are the most prevalent entity of all respiratory tract infections and mostly caused by viruses. Lower respiratory tract infections can be caused by various agents. They are the leading infectious cause of death and the fifth-leading cause of death overall. Fast Track Diagnostics (FTD) offers 18 different kits covering 39 pathogens including 25 viruses, 13 bacteria, and 1 fungus.
Objectives: To evaluate and compare the performance of FTD kits for detection of respiratory pathogens using either the VERSANT® kPCR SP (Siemens Healthineers) in combination with the VERSANT® kPCR AD (Siemens) or the QuantStudio™ 5 Dx Real-Time PCR (qPCR) System (Applied Biosystems) or the NucliSENS® easyMag® (bioMérieux) in combination with the QuantStudio™ 5 Dx Real-Time PCR (qPCR) System.
Materials and methods: For each pathogen, a dilution series was prepared before extraction. From this dilution series, 200 µl was loaded onto the NucliSENS® easyMag® platform and the remaining sample was loaded onto the VERSANT® SP module. For the nucleic acid extraction on the VERSANT® kPCR SP, the VERSANT® Sample Prep 1.0 Kit with the protocol Dynamic Assay Preparation FTD1 was used (SP1.0 500-15-10). The PCR analysis was done either on the VERSANT® AD module or on the QuantStudio™ 5 Dx Real-Time PCR (qPCR) System. For the nucleic acid extraction on the NucliSENS® easyMag®, the Generic protocol 2.0.1 was run using 200 µl sample input and 55 µl elution volume. The PCR plate was prepared manually, and the PCR was done on the QuantStudio™ 5 Dx Real-Time PCR (qPCR) System. Furthermore, Quality Control for Molecular Diagnostics (QCMD) panels were tested with the VERSANT® workflow.
Results: Overall, the VERSANT® workflows showed comparable results with both amplification instruments tested when compared to nucleic acid extraction on the NucliSENS® easyMag® platform followed by amplification on the QuantStudio™ 5 Dx Real-Time PCR (qPCR) System. For the majority of pathogens, Ct values were lower (54.4%) or equivalent (35.1%) with the VERSANT® workflows. In a minority of cases (10.5%), the NucliSENS® easyMag® workflow outperformed the sensitivity reached with the VERSANT® workflows.
Conclusions: The VERSANT® kPCR Molecular System provides high sensitivity and specificity for detection of respiratory pathogens and is suitable for use with FTD assays. Its high throughput enables laboratories to manage with high numbers of samples during seasonal peaks.

A6

Clinical Evaluation of the *EasyScreen™* Enteric Viral Detection Kit EV002

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Background: Acute gastroenteritis is the second main cause of morbidity and mortality in the developing world, with about 2 million deaths in children under the age of five recorded each year. Mortality is lower in developed countries, but morbidity and economic consequences are high. Therefore, rapid and accurate detection of enteric pathogens is vital for effective treatment.
Objectives: To evaluate the clinical performance of the *EasyScreen™* Enteric Viral Detection Kit EV002, a multiplex real-time PCR detection system.
Materials and methods: Clinical stool samples (N = 241) were sourced retrospectively from several Sydney hospital and diagnostic services. These included samples positive for norovirus GII (N = 164), norovirus GI (21), adenovirus (18, including 4 adenovirus 41), rotavirus A (10), astrovirus (7), enterovirus (3), and sapovirus (1). Furthermore, 17 negative samples were included. Nucleic acids were extracted from blinded raw stool samples using the *EasyScreen™* Sample Processing Kit and the KingFisher Flex robot in batches of 96. The eluted nucleic acids were screened using the EV002 real-time PCR kit in 384 well format. Discordants or co-infections were investigated by alternate PCR assays and DNA sequencing.
Results: For the detection of astrovirus, adenovirus, enterovirus, rotavirus A, sapovirus, and negative samples, 100% concordance between reference and EV002 was observed. In Norovirus GI samples, EV002 resulted in 95% sensitivity (20/21) and 100% specificity. Similarly, 96% sensitivity (158/164) and 100% specificity was observed for Norovirus GII. EV002 identified 25 apparent dual infections that were not tested for in the reference set. The majority (15) of these were confirmed by alternative assays and the remainder are being further investigated.
Conclusions: The *EasyScreen™* Enteric Viral Detection Kit EV002 demonstrated an overall 97% concordance in this dataset. This new kit is able to provide rapid detection of enteric viruses within 5 hours from sample to result.

A7

Screening for tropical infectious fever of unknown origin

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Background: Fever of unknown origin (FUO) remains a diagnostic challenge, as fever may be caused by hundreds of different inflammatory, rheumatic, or malignant disorders, immunotherapies, or infections. In remote tropical regions, the patient visits the doctor only for severe disease and may be infected with a rare pathogen not covered by common test panels. Multiplex NAT assays have the capability to identify a causative pathogen, help to cure patients and to prevent outbreaks. Combining panviral assays such as panFlavi (DENV, YFV, WNV, ZikaV), panAlpha (CHIKV, MayaroV, EEV/WEV), and panFilo (EBOV, MarburgV) and additional assays for LassaV, CCHFV, Plasmodium, Salmonella, Leptospira, and Rickettsia allows to cover a wide range of pathogens in one or few reactions per sample.
Objectives: To evaluate multiplex NAT assays for detection of pathogens found in tropical regions suitable for being processed in remote field laboratories.
Materials and methods: Serum samples already extracted and negative for EBOV were tested for flaviviruses, alphaviruses, filoviruses, CCHFV, and LassaV using ModularDx PCR test kits on a MyGo or a Roche LightCycler 480II instrument.
Results: Data obtained from field samples from India and West Africa including several samples from patients with suspected ebola infection will be reported.
Conclusions: Modular multiplex NAT testing with panviral assays facilitates screening large numbers of samples.

A8

Automated sample processing and detection of pathogens using the AltoStar® AM16 workflow

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Background: The AltoStar® AM16 Automation System is an IVD compliant workflow for automated sample processing and analysis by real time PCR. The AltoStar® AM16 workflow consists of the pipetting module AltoStar® AM16, the operating software AltoStar® Connect, and two real time PCR platforms CFX96 Deep Well (Bio-Rad).
Objectives: To develop an IVD certified nucleic acid extraction kit for simultaneous isolation of RNA and DNA from different clinical sample types and IVD certified real time PCR kits for detection and quantification of pathogens from clinical samples.
Material and Methods: A nucleic acid extraction kit (Purification Kit PK15-16) was designed and validated for use with the AltoStar® AM16 workflow and optimized for simultaneous isolation of RNA and DNA from various clinical matrices including plasma, serum, whole blood, urine, stool, CSF, and viral transport medium. For automated procession with the AltoStar® AM16 workflow, ready-to-use real time (AltoStar®) PCR kits for detection and quantification of CMV, EBV, BKV, JCV, and parvovirus B19 using harmonized cycling conditions and a universal internal control were developed and validated. Quantification is done using external calibrators.
Results: The Purification Kit PK15-16 allowed successful procession of RNA and DNA from clinical samples. The limit of detection and the linear range were determined for each virus detection kit: CMV (plasma) 215 IU/ml (2.5E+02 IU/ml to 1.0E+08 IU/ml), CMV (whole blood) 305 IU/ml (5.0+02 IU/ml to 1.0E+08 IU/ml), EBV (plasma) 195 IU/ml (2.0E+02 IU/ml to 1.0E+08 IU/ml), EBV (whole blood) 320 IU/ml (1.5E+03 IU/ml to 1.0E+08 IU/ml), BKV (plasma) 66 IU/ml (2.0E+02 IU/ml to 1.0E+08 IU/ml), JCV (plasma) 3.6 IU/ml (1.0E+02 IU/ml to 1.0E+08 IU/ml), parvovirus B19 (plasma) 121 IU/ml (5.0E+02 IU/ml to 1.0E+08 IU/ml).
Conclusions: The AltoStar® AM16 workflow in combination with the Purification Kit PK15-16 and the AltoStar® PCR kits allows the combination of eight different tests on a 96-well plate and 4 different tests from a single sample with high analytical performance combining the ease-of-use of automation with the flexibility needed in a diagnostic laboratory.

A9

High nucleic acids extraction efficiency from biological samples through the GENEQUALITY X120 automated platform

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Background: Molecular diagnostic laboratories require fully automated systems in order to obtain the maximum level of standardization, reliability, traceability, and efficiency. The recently introduced GENEQUALITY X120 instrument combines up to 64 simultaneous extractions from a wide range of clinical samples with automated real-time PCR plate setup.
Objectives: To evaluate the performance of the GENEQUALITY X120 Pathogen kit (AB ANALITICA) that extracts DNA and RNA simultaneously on the GENEQUALITY X120 instrument.

Materials and methods: 445 clinical samples from different matrices were tested: EDTA or citrate whole blood (28%), plasma and serum (16%), urine (19%), urogenital swabs (18%), cerebrospinal fluid, amniotic fluid, and respiratory samples (9%), cervical swabs (10%). 58% of the samples were positive for at least one viral or bacterial target. All samples were extracted on the GENEQUALITY X120 instrument using the GENEQUALITY X120 Pathogen kit. Reference methods included the NucliSENS® easyMAG platform (bioMérieux) and the EZ1 Advanced XL (QIAGEN) instrument using the EZ1 DSP Virus kit. In addition, 90 EQA QCMD samples from 2013, 2015, and 2016 panels were analyzed. For evaluation of potential cross reactivity and interference effects, biological samples spiked with substances such as hemoglobin, bilirubin, lipids, and heparin were used. All the samples were amplified with REALQUALITY qPCR assays (AB ANALITICA).
Results: Results for all clinical and QCMD samples tested with the GENEQUALITY X120 Pathogen kit on the GENEQUALITY X120 instrument were found concordant when compared to the reference methods. No interference was observed regarding extraction efficiency caused by endogenous substances, except of heparin. Cross-contamination tests confirmed the absence of cross reactivity. Repeatability tests showed an intra-assay variability coefficient of <1.5% and an inter-assay coefficient of <2.6%.
Conclusions: This study demonstrates that the GENEQUALITY X120 Pathogen kit, in combination with the GENEQUALITY X120 instrument, fulfills all requirements for an efficient use in the routine diagnostic laboratory.

A10

Torque Teno Virus: a clinically relevant immune status biomarker for immunocompromised patients?

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Graft success after transplant surgery requires balance of immunosuppression to prevent organ rejection but also opportunistic infections. Torque Teno Virus (TTV) is a DNA virus, belonging to the *Anelloviridae* family. TTV is a non-enveloped, single-stranded, circular DNA virus carried by nearly everyone. To date, no clinical pathology or clinical symptoms are directly associated with this virus. Recent studies have shown that TTV viral load can provide a picture of a person's immune status. TTV viral load has been demonstrated as being predictive for the development of infections in lung transplant and stem cell transplant recipients and has also been associated with prediction of organ rejection in different transplant settings. This presentation will give a review of the literature regarding the knowledge on TTV and its potential use as a biomarker in different transplant settings (mainly in lung, kidney, and stem cell transplantation). TTV viral load may be used to monitor immunosuppression therapy for transplant recipients to achieve the individual balance for optimal immunosuppression.

A11

Validation and performance of a comprehensive lung cancer NGS assay for a therapeutic mutation survey in a Lower Austrian molecular pathology laboratory

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Background: An increased number of diagnostic genes with approved treatment and the diversity of targetable mutation types, including gene fusions and copy number variants (CNVs), represent major issues for next generation sequencing (NGS) applied to optimization of lung cancer patient therapy.
Objectives: To establish and validate a comprehensive NGS lung cancer gene panel for the clinical routine laboratory.
Materials and methods: DNA and RNA were purified from 112 lung cancer samples (FFPE tissue sections or GIEMSA-stained cell preparations) with a QIAcube-automated AllPrep DNA/RNA FFPE Kit® (Qiagen). Nucleic acid quality was assessed by Fragment Analyzer Automated CE System® assays. NGS library preparations and sequencing on a MiSeqDx® instrument were performed with the TruSight Tumor 15® (TST15®; Illumina) and the Archer Comprehensive Thyroid and Lung Cancer® (CTL, ArcherDx®; DNA: VariantPlex® CTL, RNA: FusionPLex® CTL) gene panels. Reference materials were from Horizon® (Structural Multiplex Reference Standard FFPE®) and Seracare® (Seraseq™ FFPE Tumor Fusion RNA Reference Material v2). Three independent bioinformatic pipelines (VariantStudio® 3.0, SeqNext® 4.4.0, Archer® Analysis 5.1.2) were used for data analysis.
Results: Parallel mutation detection in hotspot regions of the AKT1, BRAF, EGFR, ERBB2, KRAS, NRAS, PIK3CA, RET, and TP53 genes with the in house validated TST15® NGS panel and the VariantPlex® CTL kit gave identical results in all patient samples. Although Archer® assays use a different amplification technology (Archer® Anchored Multiplex PCR Chemistry, AMP™) together with molecular barcodes mutated allele frequencies were comparable. Treatable EGFR and MET gene amplifications were assessed with the Archer® kit. The VariantPlex® CTL assay (100 ng DNA) detected all mutations in the Structural Multiplex Reference Standard FFPE® panel (n = 3). The FusionPlex® CTL (100 ng RNA) assay found all 14 gene fusions and oncogenic isoforms (inclusive MET exon 14 skipping) in the Seraseq™ control (n = 3).
Conclusions: The Archer Comprehensive Thyroid and Lung Cancer® panel is a reliable gene analysis tool for the molecular pathology laboratory. Cytological lung aspirates or tiny biopsies with low total DNA amounts can be analyzed with the TST15® gene panel (1 to 10 ng DNA/reaction).

A12

Validation of a new commercial assay for detection of *SLCO1B1* allelic variant c.521T>C by real-time PCR using melting curve analysis

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Background: *SLCO1B1*, a liver specific organic anion transporter protein, plays an important role in clearing statins from blood. Statins are frequently prescribed drugs for treatment of hypercholesteremia and prevention of cardiovascular disease. Adverse effects have been reported in many cases due to the high number of prescriptions. The most common form is myopathy; however, in rare cases, rhabdomyolysis with fatal outcome may develop. Multiple SNPs have been identified in the *SLCO1B1* gene and the variant c.521T > C has been found to have a strong association with simvastatin-induced myopathy.
Objectives: To develop a real-time PCR-based assay to test for the *SLCO1B1* c.521T > C (V174A) allelic variant on a LightCycler® glass capillary based system using melting curve analysis.
Materials and methods: Real-time PCR was performed using genomic DNA isolated from 126 clinical specimens. The new *SLCO1B1* c.521T > C assay was applied on two different LightCycler® instruments (with 3 and 6 channels). As reference method, another commercial *SLCO1B1* c.521T > C assay using TaqMan chemistry and Sanger sequencing was used.
Results: Genotyping results were found to be independent of the real-time PCR instrument used. Furthermore, a 100% agreement was observed between LightCycler® instruments using melting curve analysis and the TaqMan based system. No discrepant results were seen when comparing real-time PCR and DNA sequencing data.
Conclusions: The new real-time PCR based *SLCO1B1* c.521T > C assay was found to be fully compatible with both of the LightCycler® instruments. This test represents a useful tool to identify patients unlikely to develop adverse effects and benefit from a high dose statin therapy as well as heterozygous or homozygous carriers with raised myopathy risk requiring adjustment of simvastatin dosage.

Pathogens – HDV / Preparedness for Public Health Emergencies (A13 – A16)

A13

Reliability of HDV RNA quantification: where are we?

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Background: Nucleic acid amplification technique (NAT)-based tests to detect HDV RNA are the most sensitive tool for HDV diagnostics. The main clinical utility for quantification of HDV RNA is to monitor antiviral therapy of patients. Furthermore, individuals with an active HDV infection can be identified by NAT testing providing a more real picture of the HDV prevalence in the HBsAg positive population. As most assays for the detection of HDV RNA are not standardized, comparison of test results remains difficult.
Objectives: To improve interlaboratory results for the detection and quantification of HDV RNA, a candidate World Health Organization (WHO) International Standard (IS) was developed and evaluated in a collaborative study. Additional studies were performed to demonstrate that the HDV RNA values measured for the IS, representing genotype 1, and for clinical samples do have the same relationship between two or more HDV NAT tests (commutability of the IS).
Materials and methods: To address the commutability issue of the WHO reference material, two EQA HDV study programs were designed: The 12-member panel PEI multicenter study (5 sites, 7 HDV NAT assays) included 10 different neat HDV positive plasma samples and 2 replicate samples of the IS, while the Quality Control for Molecular Diagnostics (QCMD) HDV EQA program included dilutions of 2 different HDV positive plasma specimens derived from HDV genotype 1 and the IS (target concentration of 4.00 $\log_{10}$ IU/mL). Potencies of the samples were estimated relative to assigned unitage of the IS.
Results: Results obtained from 7 different quantitative real-time NAT tests were analyzed by pair-wise test comparison. Regarding HDV clinical samples, few NAT tests showed poor correlation but the majority of results obtained harmonized very well within most of the assays and the IS results fitted within the CI of the regression line demonstrating commutability. The QCMD HDV pilot program started in 2014 with 20 participants using quantitative NAT tests. In 2017, the number of participants had increased by 70 percent. Comparison of quantitative results obtained over 4 years revealed remarkable differences of concentrations reported as a result of different test design and insufficient calibration. Performance data for both HDV positive specimens were slightly different indicating intragenotype 1 sequence diversities. Nevertheless, the calculated potency for both HDV positive plasma samples relative to the assigned potency of the IS showed a marked improvement in the agreement between the majorities of the used tests and these data provide further evidence for commutability of the IS.

Conclusions: Results from both study programs demonstrated that the WHO standard is suitable to harmonize HDV NAT assays. Reliable HDV RNA detection and quantification can only be achieved by a carefully designed assays, together with a test calibration using the standard as reference material.

A14

Clinical application of droplet digital PCR for hepatitis delta virus RNA quantification

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Background: Droplet digital PCR (ddPCR) is a third generation PCR technology that enables the absolute quantification of target nucleic acid molecules. Several recent studies highlight its potential clinical utility for viral diagnostics applications.
Objectives: To develop a ddPCR assay for hepatitis delta virus (HDV) RNA quantification and to evaluate its performance in comparison to real-time PCR assays.
Methods: Primers and probe were designed from conserved regions of aligned sequences of 8 different HDV genotypes. Accuracy and linear range were assessed analyzing ten-fold dilution series of the WHO HDV International Standard. The limit of detection was determined by Probit analysis. Intra- and inter-assay variabilities were determined by performing triplicate experiments at different viral load levels. To evaluate the clinical performance, 110 human plasma specimens collected from 50 patients with chronic hepatitis D were tested with ddPCR and results were compared to those obtained with two one-step real-time PCR assays.
Results: By linear regression analysis of serial dilutions of the WHO reference material, ddPCR showed a good linearity with a slope coefficient of 0.966 and an R^2 of 0.980. The conversion factor between copies/ml and IU/ml was 0.97 and the new assay showed an analytic measuring range between 10 and $1x10^6$ IU/ml. Data obtained from Probit analysis confirmed the high sensitivity of the test; the limit of quantification was found to be 9.2 IU/ml and the limit of detection 1.1 IU/ml. The assay showed an intra-assay CV of 0.80 and an inter-assay CV of 1.51. When the clinical performance of the new ddPCR assay was evaluated, results obtained exhibited a good degree of correlation with both a one-step real-time "in-house" assay ($R^2 = 0.899$) and a commercial kit ($R^2 = 0.840$). Moreover, a high correlation was also observed when 45 consecutive plasma samples of 9 patients treated with interferon were analyzed ($R^2 = 0.946$).
Conclusions: ddPCR for HDV RNA detection and quantification shows a technical and clinical performance comparable to that of both of the two one-step real-time assays used in this study. ddPCR, providing absolute quantification without relying on a standard curve and on PCR amplification efficiency, may represent a viable alternative to real-time PCR for the assessment of plasma HDV viremia.

A15

Comparison of automated extraction and amplification platforms on quantification of plasma HDV RNA

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Background: Quantification of plasma HDV RNA is the essential tool for monitoring the effectiveness of antiviral therapy. For this purpose, the CE-IVD marked RoboGene® HDV RNA Quantification Kit 2.0 can be applied in combination with a manual RNA extraction procedure to obtain reliable quantification results. By now there is growing demand for automated nucleic acid (NA) extraction on various platforms. Therefore, a study for the evaluation of different extraction devices in combination with different real-time PCR systems using the RoboGene® HDV Kit for HDV RNA load determination will be performed in several diagnostic labs.
Objectives: To evaluate the RoboGene® HDV Kit in several European routine diagnostic laboratories using different automated NA extraction platforms in combination with different amplification instruments. To calibrate different combinations against the 1st WHO standard for HDV RNA using the RoboGene® HDV assay in order to harmonize quantification results obtained. To assess analytical sensitivities by determining the limit of quantification (LOD) and the linear range of quantification. To compare HDV RNA concentrations obtained with reference material and clinical specimens throughout all participating laboratories.
Materials and methods: Experiments for assay calibration and performance assessment will be carried out by using the 1st WHO standard for HDV RNA and blinded, left-over specimens (positives and negatives). All HDV-positive samples will be further diluted in HDV negative plasma and all specimens will be provided equally to each laboratory participating. Depending on the participating laboratory, automated HDV RNA extraction will be performed on MagNA Pure 96 (Roche), QIAsymphony (Qiagen), eMAG® (bioMerieux), EZ1 Advanced XL (Qiagen), or InnuPure C16 *touch* (Analytik Jena) using appropriate RNA extraction kits. Subsequent HDV RNA amplification and quantification will be assessed on Rotor-Gene® Q (Qiagen), LightCycler® 480 II (Roche), CFX96™ (Bio-Rad Laboratories), 7500 (Applied Biosystems), or qTOWER^3 (Analytik Jena) real-time PCR instruments using the RoboGene® HDV RNA Quantification Kit 2.0. Manual HDV RNA extraction applying INSTANT Virus RNA/DNA Kit (Analytik Jena) and HDV RNA load determination using RoboGene® HDV RNA Quantification Kit 2.0 on Rotor-Gene Q or LightCycler® 480 II will be performed for comparison of results obtained. In order to

further evaluate and compare HDV RNA quantification in clinical samples, the QCMD panel QAV144170_1 consisting of 8 to 10 members will be analyzed by each participating lab.
Results: This study is scheduled to start in March 2018 and to end in December 2018. First results of assay calibration and LOD determination using the 1st WHO standard for HDV RNA will be presented.
Conclusions: Harmonization of quantification results throughout all participating laboratories using particular combinations of automated extraction platforms and amplification instruments with the CE-IVD marked RoboGene® HDV RNA Quantification Kit 2.0 will be achieved through calibration against the 1st WHO standard for HDV RNA.

A16

Responding to public health emergencies - preparedness planning by the World Health Organization

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Background: The Ebola outbreak in West Africa (2014/15) showed that the route of an outbreak may take in countries characterized by weak health systems and by lack of preparedness to potential health disasters. WHO identified the need for preparedness to potential future outbreaks, covering the fields of diagnostics, vaccines, and therapeutics.
Objectives: To establish roadmaps for research and development against specific high priority pathogens covering the different fields of intervention by target product profiles. To prepare a list of high priority pathogens with the potential for future outbreaks.
Materials and methods: Roadmaps are being developed for individual pathogens and interventions, with inclusion of platform technologies as far as possible. Potential regulatory challenges for the different products are considered in advance.
Results: Taking MERS CoV as an example, the landscape analysis of diagnostics identified numerous assays, with point of care devices still being needed for surveillance. Several vaccines at different stages of preclinical development are already in the pipeline, with camel vaccines as a potential option for controlling the spread of the virus into humans. Therapeutics for MERS CoV cover both a number of monoclonal antibodies at different stage of preclinical assessment and convalescent plasma.
Conclusions: Preparedness for potential future outbreaks includes both research and development in the specific fields of high priority pathogens. In addition to individual roadmaps being developed for the products needed, regulatory preparedness has to be taken into consideration.

Pathogens – HIV and other Sexually Transmitted Infections / Gastrointestinal Viruses (A17 – A20)

A17

Tobacco use enhances immune activation but impairs immune function in HIV patients

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Background: The influence of tobacco smoking on the immune system of HIV infected individuals is largely unknown. We investigated the impact of tobacco smoking on immune activation, microbial translocation, immune exhaustion, and T-cell function in HIV infected individuals.
Objectives: To analyze association of smoking on the immune system and function in patients with HIV infection, 25 smokers and 25 non-smokers with HIV infection were compared to 15 smokers and 15 non-smokers without HIV infection.
Materials and methods: HIV infected smokers and non-smokers with documented viral suppression on combination antiretroviral therapy and HIV uninfected smokers and non-smokers were enrolled. Markers of immune activation (CD38 and HLA-DR) and immune exhaustion (PD1, Tim3, and CTLA4) were analyzed in peripheral blood mononuclear cells (PBMCs) by flow cytometry. Plasma markers of microbial translocation, soluble-CD14 (sCD14) and lipopolysaccharide (LPS), were measured. Antigen specific functions of CD4+ and CD8+ T-cells were measured by flow cytometry in PBMCs after 6 hours of stimulation with a cytomegalovirus, Epstein-Barr virus, and influenza virus (CEF) peptide pool.
Results: Compared to non-smokers, smokers of HIV infected and uninfected groups showed significantly higher CD4+ and CD8+ T-cell activation with increased frequencies of CD38+HLA-DR+ cells with a higher magnitude in HIV infected smokers. Expressions of immune exhaustion markers (PD1, Tim3, and CTLA4) either alone or in combinations were significantly higher in smokers, especially on CD4+ T-cells. Compared to HIV uninfected non-smokers, microbial translocation (sCD14 and LPS) was higher in smokers of both groups and directly correlated with CD4+ and CD8+ T-cell activation. Antigen specific T-cell function showed significantly lower cytokine response of CD4+ and CD8+ T-cells to CEF peptide-pool stimulation in smokers of both HIV infected and uninfected groups.

Conclusions: Our results suggest that smoking and HIV infection independently influence T-cell immune activation and function and together they present the worst immune profile. Since smoking is widespread among HIV infected individuals, studies are warranted to further evaluate the cumulative effect of smoking on impairment of the immune system and accelerated disease progression.

A18

Vascular health in HIV: the role of cardiovascular risk factors, biomarkers of inflammation, HIV disease status and antiretroviral therapy (results from the EndoAfrica study, Cape Town, South Africa)

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Background: HIV infection and antiretroviral therapy (ART) are associated with increased cardiovascular risk and endothelial dysfunction. However, little is known about the cardiovascular risk and disease in sub-Saharan Africa, the epicenter of the global HIV epidemic.
Objectives: To assess whether HIV infection and ART are associated with vascular changes and cardiovascular risk.
Methods: The cross-sectional data form part of the EndoAfrica study in the Western Cape, South Africa. Study participants were recruited from health clinics between 2015 and 2017 and allocated to HIV-negative (HIV-), HIV-positive ART naïve (HIV+ART-) and HIV-positive with ART (HIV+ART+) groups. Medical history, anthropometric measurements, cardiovascular examination, biochemical analyses, and vascular assessments (flow-mediated dilatation (FMD) of the brachial artery, carotid intima-media thickness (IMT) and retinal microvascular caliber measurements) were obtained.
Results: The cohort consisted of 427 participants (HIV- 148, HIV+ART- 69, and HIV+ART+210), of whom 69% were female with a mean age of 39.4 years. The cohort showed a high incidence of cardiovascular risk factors: visceral obesity (53.4%), smoking (63.4%), diastolic hypertension (32.6%), low HDL-cholesterol (32.9%), and high LDL-cholesterol (28.1%). Furthermore, there was a high incidence of cardiovascular disease biomarkers: high CRP: 70.8% and high albumin-creatinine ratio (ACR): 21.8%. Mean HDL-cholesterol levels were lower in HIV+ART- compared to HIV- and HIV+ART+, and the HIV+ groups showed increased mean ACR levels. The HIV+ART+ group had significantly reduced viral load (VL) levels compared to HIV+ART-. FMD was reduced in HIV+ART- (5.6%) vs. HIV- (6.0%) and HIV+ART+ (7.4%) ($p = 0.04$), the incidence of endothelial dysfunction (median FMD) was lower in HIV+ ART+ (43.4%) vs. HIV+ART- (57.6%) and HIV- (55.9%) ($p = 0.02$), and Central Retinal Venular Equivalent (CRVE) was lower in HIV+ART+ (226.7µm) vs HIV+ART- (237.2µm) and HIV- (233.7µm) ($p < 0.01$). ACR was a negative predictor of FMD (β: -0.16; $p < 0.01$) in the whole cohort and in the HIV+ group. In HIV-infected participants, ART was positively associated with FMD (β: 0.17; $p = 0.03$); however, CD4 and VL were not associated. HIV-parameters were not associated with either IMT or Central Retinal Arteriolar Equivalent. In HIV+ participants, ART negatively predicted CRVE (β: -0.15; $p = 0.01$), whereas VL was positively associated with CRVE (β: 0.2; $p < 0.01$). Furthermore, the odds of presenting with endothelial dysfunction was lower with ART (OR: 0.25; $p = 0.04$).
Conclusions: This cohort was characterized by a high cardiovascular risk profile. From the data, it appears that ACR may be regarded as an important biomarker of vascular function in HIV. Finally, ART appeared to be vasculo-protective as shown by reduced endothelial dysfunction and CRVE in the HIV+ART+ group.

A19

Multi-target PCR – the multi-tool in molecular diagnostics?

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Background: Several gastric, respiratory, and sexually transmitted infections are caused by a variety of bacteria and viruses. Future studies must evaluate the relevance of multiple infections for the clinical outcome of the patient. Efficient and reliable multi-target PCR tools to investigate clinical samples with a high output of information and minimal laboratory hands-on-time are required. Recently, sexually transmitted infections multi-target PCR assays (Allplex™ STI Essential and Allplex™ Genital Ulcer, Seegene Inc.) have been brought on the market. The assays allow identification of 13 pathogens in two separate PCR reactions. Pathogens detected include *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *T. vaginalis*, *U. urealyticum*, *U. arvum, T. pallidum*, *H. ducreyi*, HSV-1, HSV-2, VZV, and CMV.
Objectives: To present results obtained by the Allplex™ STI Essential and Allplex™ Genital Ulcer assays.
Materials and methods: Clinical samples were obtained from routine diagnostic workflow. Urine specimens and genital swabs collected from different patient cohorts were processed using the automated Microlab Nimbus® instrument followed by an automated PCR setup. Amplification and detection was conducted using the CFX96 thermocycler (BioRad, USA) in combination with the SeegeneViewer® software.

Results: With the multi-target PCR, a high number of coinfections could be detected. Up to five different pathogens were detected simultaneously. Samples from cohorts with an elevated risk for sexually transmitted infections revealed a high percentage of patients infected with more than one pathogen.
Conclusions: Results demonstrate the benefit of multi-target PCR tools for the diagnosis of infectious diseases. The assays evaluated show a valid and reproducible performance. They are potentially useful tools for detecting multiple infections in laboratories where the daily sample number varies between low- and high-throughput batch testing, due to a flexible automation concept.

A20

Update on intra-individual norovirus evolution: genotypic and phenotypic analyses

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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 7 patients analyzing 26 samples.
Materials and methods: The 2-4 longitudinal specimens covered 18-470 days. Capsid protein was expressed recombinant and binding to autologous serum antibodies was measured. Amplification products comprising 760 nt of P2 domain (454) and 1623 nt of the capsid gene (PacBio) 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 and capsid protein structures were reconstructed by molecular dynamics analysis.
Results: Variable sites between consensus sequences correspond to previous reports and putative epitopes. Number of reconstructed quasispecies tended to increase at the onset of infection and decrease in later stages. Capsid-specific antibodies increased over time. Mutations correlated with decreased antibody binding to capsid proteins. Longitudinal mutations lead to changes of predicted capsid conformations.
Conclusion: Fast evolution of the capsid gene in chronically infected patients impacts antibody binding and protein conformation.

Liquid Profiling / Molecular Oncology (A21 – A23)

A21

Next generation sequencing in molecular hemato-oncology

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Background: Next-generation sequencing (NGS) has uncovered the genomic landscape of many types of cancer. A number of recurrent somatic mutations has been incorporated in the latest WHO classification of hematologic malignancies, and testing in patient samples has become a powerful tool to support diagnostic decisions, to improve prognostic classification, and to identify potential treatment targets. Despite the high clinical potential of this technique, a number of issues need to be taken into consideration when performing NGS-based diagnostics in hemato-oncology. First, most applications of NGS represent a typical in-house test, and evaluation of performance characteristics by the laboratory is warranted. In addition, somatic mutations reflecting clonal hematopoiesis are also in a significant number of apparently healthy, mostly elderly individuals – a condition currently referred to as clonal hematopoiesis of indeterminate potential (CHIP).
Objectives: To evaluate the performance characteristics of targeted NGS panels for myeloid malignancies.
Materials and methods: Cell lines with known variant allele frequency of somatic mutations (HorizonDx) were used, together with primary patient samples to validate the assays. Overall, 27 patients diagnosed with myeloid neoplasms and mutations confirmed by PCR or Sanger sequencing were included. Library preparation was done using either the TruSight Myeloid Panel (Illumina) or the Myeloid Solution Panel (Sophia Genetics), libraries were sequenced on a MiSeq instrument (Illumina), and data were analyzed using the respective bioinformatics-pipelines (Sophia Genetics DDM Pipeline, Illumina Variant Studio). Reproducibility was determined by multiple inter- and intra-assay comparison in three independent library preparations.
Results: Analysis of control material identified a variant allele frequency of 2% as limit of detection. At this cutoff, the error rate of the Myeloid Solution Panel (Sophia Genetics) was superior. In the patients' cohort, all known mutations were identified with a sensitivity >99% and a specificity >99%. Inter- and intra-assay reproducibility was >99%.

Conclusions: The performance characteristics of NGS-based molecular testing is sufficient for diagnostic application. Large gene panels can be used for a number of different hematologic neoplasms and, thus, streamline the diagnostic workflow. Genetic characterization of cancer samples will become even more import and will augment or even replace current morphology-centered classification systems. Nevertheless, somatic mutations in hematopoietic cells are also found in CHIP. Thus, the integration of morphology, immunophenotyping, cytogenetics, and molecular-genetics remains the diagnostic gold standard in hemato-oncology.

A22

Liquid profiling for the detection of RAS mutations - facts and fiction

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Non-invasive analysis of circulating tumor DNA (ctDNA), referred to as liquid profiling, is a promising application in personalized cancer therapy. We evaluated a commercially available diagnostics system for the detection of RAS mutations (OncoBEAM RAS CRC kit, Sysmex) in patients with advanced colorectal cancer. Liquid biopsy is able to provide information regarding therapy response, cancer relapse, and mechanisms of resistance. In contrast to standard biopsy, liquid profiling can be performed repeatedly and thus allows evaluation of metastasis and monitoring of the actual treatment response in real-time. Nevertheless, the low number of samples that can be analyzed simultaneously as well as the restriction to advanced cancer stage currently limits the field of application of the OncoBEAM technology for liquid profiling in routine. The use of this technology for early detection of cancer is currently under investigation.

A23

Bioinformatics tools in hematological diagnostics and research and their application to whole genome sequencing

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Background: In the last years, next generation sequencing (NGS) has largely replaced traditional Sanger sequencing not only in research but also in routine diagnostics. Therefore it is now possible to detect mutations in large gene panels for many patients with short turnaround times. Dedicated bioinformatics tools are indispensable in order to handle and interpret the large amount of data generated by NGS technologies and this will become even more challenging as research and diagnostics move into the realm of whole genome sequencing (WGS) and RNA sequencing (RNASeq).
Objectives: To sequence 5,000 whole genomes and transcriptomes of patients with a wide range of hematological malignancies.
Materials and methods: The NGS data generated by the MLL5k project allows for the generation of gene expression profiles and the detection of a wide array of mutations, including small scale variation (single nucleotide variants, insertions/deletions) and larger scale aberrations (copy number variation, structural variants). Data are explored with regard to the hematological entities included and with a variety of approaches. Furthermore, data are compared to the results of routine diagnostics based on current gold standard methods.
Results: Explorative gene expression analysis corroborated the separability of hematological entities but also revealed a high diversity within these entities which might suggest more complex regulations as indicated by contemporary methods. Additionally, the WGS approach for structural variation analysis allows for detection of diagnostically significant chromosomal fusions as well as resolving complex rearrangements at higher resolution than current standard techniques such as chromosome banding analysis.
Conclusions: With WGS and RNASeq becoming standard approaches in hematological research, they also have the potential of becoming routine diagnostic tools in the future, complementing or even replacing established laboratory methods.

Pharmacogenetics – New Approaches (A24 – A26)

A24

Multi-modal decision support to enable pharmacogenetics-based drug dosing across seven European countries

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Background: While clinical pharmacogenomics (PGx) has emerged as a promising approach to improve the safety and efficacy of pharmacotherapy by stratifying drug dosing and drug selection based on genetic patient data, its widespread adoption is significantly dependent on its integration into routine care via clinical decision support (CDS) tools. Previous implementation efforts were predominantly characterized by the need for a sophisticated and interconnected health IT infrastructure, which is, however, unfortunately still unavailable in many healthcare settings.
Objectives: To develop a unique multi-modal CDS concept consisting of digital, paper-based, and mobile tools that enables the delivery of PGx CDS given a wide variety of technical framework conditions and to implement it in the context of the Ubiquitous Pharmacogenomics (U-PGx) project.
Materials and methods: More than 15 clinical sites across seven European countries with largely varying health IT infrastructures, ranging from complete absence of any such infrastructure to full integration of CDS alerts in the electronic health record, are participating in this project.
Results: U-PGx Genetic Information Management System (GIMS) developed by the U-PGx partner bio.logis Genetic Information Management GmbH enables clinical sites to upload anonymized genetic samples and retrieve PGx test results and individualized recommendations authored by the Dutch Pharmacogenetics Working Group for 40 drugs associated with one or more of 12 different genes. PGx results and dosing recommendations in the respective local languages are retrievable in the following optional formats: (1) Semi-structured data for incorporation into local electronic health records, (2) a digital PGx report format that can be filed in the patients' digital or paper-based health records, (3) a 'safety-code' card that is part of a mobile-based CDS system and allows for the retrieval of patient-specific results via a smartphone or tablet. As of February 2017, our CDS tools have been successfully rolled out at participating sites in three countries, i.e. Greece, Slovenia, and Spain. Roll-out at institutions in the UK, the Netherlands, Austria, and Italy will be commenced in summer 2018. Preliminary evaluation results showed good acceptance of our CDS tools among clinicians.
Conclusions: Utilizing a unique and new multi-modal CDS concept that accommodates the diversity of IT infrastructures commonly encountered in healthcare settings, a viable model to advance the integration of PGx into clinical practice has been developed.

A25

ABCB1 test: blood-brain barrier pharmacogenetics for antidepressant therapy

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Background: Depression is one of the most serious mental disorders estimated to affect about 350 million people worldwide. Less than one third of patients suffering from severe depression experience complete remission when initially treated with antidepressant agents. A key factor for the effectiveness of antidepressants is the ability to pass the blood-brain barrier, which is regulated by transporter molecules such as the P-glycoproteins (PgP), encoded by the ABCB1 gene.
Objectives: To evaluate the clinical utility and performance of a new diagnostic assay, RIDA®PRECISION ABCB1 real-time PCR assay, for the detection of ABCB1 gene variants in human whole blood samples.
Methods: November 2016 through February 2018, a total of 1121 patients were referred to laboratory Viollier for ABCB1 pharmacogenetic testing. EDTA blood was extracted using the Maxwell® RSC Whole Blood DNA kit (Promega) with the Maxwell® RSC Instrument (Promega). Extracted nucleic acids were analyzed with the RIDA®PRECISION ABCB1 real-time PCR assay on the LightCycler® 480II (Roche) to detect two ABCB1 SNPs (rs2032583, rs2235015) that associate different treatment outcomes.
Results: Overall, 857 patients (76.4%) with ABCB1 gene variants were identified which, according to their presence or combination of SNPs, required a more intense treatment plan: (1) increasing the dosage of the PgP substrate or (2) treatment with a PgP non-substrate or (3) application of augmentation using anticonvulsants, lithium, or atypical antipsychotics and psychotherapy. The remaining 264 patients (23.6%) were identified as carrier of the CC/CT genotype at SNP rs2032583 and the TT/GT genotype at SNP rs2235015. According to previous research, these patients require treatment with a PgP substrate and the dose administered results in plasma drug concentrations within the recommended limits for a beneficial treatment outcome.
Conclusions: Three out of 4 patients carry gene variants encoding for PgP that lead to decreased passage through the blood-brain barrier. The RIDA®PRECISION ABCB1 real-time PCR assay may lead to faster treatment success and predict which antidepressants are best suited for the individual patient. Future studies are required to correlate the diagnostic results with patient outcome.

A26

Applications of pharmacogenetics (PGx) in a clinical setting

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Background: Pharmacogenetics (PGx), the study of inherited genetic differences in drug metabolic pathways which can affect individual responses to drugs, has been known for 50 years. Today, the "plateau of productivity" (Gartner Hype Cycle) is reached offering a personalized medicine due to new technical and scientific possibilities ranging from large scale PGx analysis to result interpretation by databases for "clinical decision support (CDS)".
Objectives: To introduce the MassARRAY® System by Agena Bioscience™ (Agena Bioscience™, San Diego, CA, USA), a high throughput PGx system allowing professional interpretation of results, in a routine diagnostic laboratory.
Materials and methods: The MassARRAY® System by Agena Bioscience™ is based on multiplex PCRs, followed by single base primer extension, automated post-PCR sample processing, and a high sensitive detection by the time-of-flight technique. The manufacturer offers a PGx gene panel based on CIPC® (https://cpicpgx.org/) dosing guidelines including CYP (CYP2D6 amplification testing by paralogues), ABCB1, DPYD, TPMT, OPRM1,SLCOB1, and MTHFR. For PGx results interpretation regarding multiple drug therapy, a PGx database including CDS provided from SONOGEN AG (Zurich, Switzerland) was used.
Results: DPYD testing (F-Fluorouracil/capecitabine toxicity) showed DPYD*2A/*2B in 4 of 208 cancer patients (1.9%)., In 1/4 cancer patients, a DPYD *1/*1 EM was detected. Additionally, for CYP2D6, a *1/*1xN UM status for ondasentron was found using the PGx panel in this patient.
Conclusions: Introducing PGx using the new MassARRAY® System by Agena Bioscience™ in combination with a PGx database including CDS provided from SONOGEN AG allows to improve personalized medicine.

Poster Session (P1 – P17)

P1

The novel *Tropheryma* species is rarely found in clinical specimens

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Background: Very recently, a novel *Tropheryma* species (TS) has been described in a kidney transplant recipient with recurrent pleuritis [1]. Its 16S rDNA, a frequent target for PCR assays to detect *T. whipplei* (TW), shows only 98% homology to that of TW.
Objectives: To investigate whether our diagnostic PCR for detection of TW would also detect TS and, if yes, whether TS can be found among our TW-positive clinical specimens (mainly stools).
Materials and methods: *In silico* analysis showed that primer/probe sequences of the currently used real-time PCR assay differ in only one position of the forward primer from the sequence of TS whereas reverse primer and FRET-probes are homologous to TS. However, in the region amplified, three nucleotides are different between the two species allowing differentiation by sequencing. Twenty-four clinical specimens previously positive in the routine TW-assay were amplified in parallel using a forward primer complementary to either TW or TS.
Results: Nineteen specimens tested positive with both assays showing almost identical Ct values, two tested positive only with the TS primer and three only with the TW primer. The latter five showed very high Ct values in the original analysis and were thus very close to the limit of detection. When amplification products of 19 specimens were sequenced in both directions, none of the three nucleotides characteristic for TS were found. All sequences were identical to those of TW strain Twist.
Conclusions: The newly described *Tropheryma* species was not present in specimens from patients with suspected Whipple's disease sent to laboratory Bioanalytica AG.

[1] Vankeerberghen A., Jonckheere S., De Raeve H., Caluwe R., De Beenhouwer H. Clin Microbiol Infection 2017; doi: https://doi.org/10.1016/j.cmi.2017.09.011

P2

Prenatal *RHD* genotyping: automated extraction of cell-free fetal DNA using the QIAsymphony SP platform

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Background: Diagnosis of fetal sex, Rh blood group/D antigen (*RHD*) genotype, and chromosomal abnormalities can be achieved only through analysis of fetal DNA. Studies on cell-free fetal DNA (cffDNA) in maternal plasma allow noninvasive prenatal testing.
Objectives: To evaluate automated cffDNA extraction using the QIAsymphony SP platform (Qiagen, Hilden, Germany) and to compare the prenatal with the postnatal *RHD* genotype.
Materials and methods: Eighty-three *RhD*-negative women, week 12 – 30 of gestation, followed up at hospitals in Croatia, were included in this study. The QIAsymphony SP DSP Virus Midi Kit (Qiagen) was employed for extraction of cffDNA from maternal plasma according to the manufacturer's instructions using the QIAsymphony SP platform. The input volume was 1000 µl and the elution volume was 60 µl. Automated extraction was done in duplicate. Fetal fragments of exons 7 and 10 of the *RHD SRY* genes were amplified in triplicate by RT-PCR on the Applied Biosystems 7500 real time (RT) PCR System (Applied Biosystems, Foster City, CA, USA). In case of an *RHD*-negative female fetus, fragments of *RASSF1A* and *β-actin* genes were amplified. The maternal *RASSF1A* fragment was digested with the *BsTUI* restriction enzyme with the non-cleaved part representing fetal DNA. The *β-actin* gene fragment served as internal control of digestion efficiency because the *BsTUI* enzyme digests both, maternal and fetal DNA.
Results: Comparison with the postnatal *RhD* status showed full concordance with the determined *RHD* genotype. The test yielded 100% sensitivity, specificity, and accuracy, respectively.
Conclusion: Extraction of cffDNA can be automated successfully using the QIAsymphony SP platform employing the QIAsymphony SP DSP Virus Midi Kit.

P3

A real-time multiplex-PCR for the detection of extended-spectrum β-lactamases (ESBL) and carbapenemase-producing organisms (CPO) using 3base™ technology

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Background: Beta-lactam and carbapenem antibiotics are the most commonly used worldwide in the treatment of bacterial infections. The recent emergence of ESBL and CPO is a significant global concern in healthcare settings, as standard treatments may be rendered ineffective. Thus accurate and rapid detection of these resistant organisms will have a significant impact on patient management.
Objectives: To develop a real-time multiplex-PCR assay for detection of the most significant and commonly encountered bacterial resistance genes TEM, CTX-M, SME, GES, IMP, NDM, OXA 23, OXA48, OXA51, MCR-1, DHA, SHV, VIM, IMI, CMY, KPC and their subtypes.
Materials and methods: Synthetic DNA constructs were designed to test the assay sensitivity. In addition, validation organisms and panels were obtained from Vircell, Zeptometrix, and QCMD to test the performance of the assay on known reference material. Over 130 clinical isolates were obtained from St. Vincent's Hospital (Sydney, Australia) and University Hospital Galway (Galway City, Ireland). DNA extraction and PCR setup were performed on a Hamilton Nimbus platform, especially modified for Genetic Signatures, PCR amplification and detection on a CFX384 Touch™ (Bio-Rad) real-time PCR instrument.
Results: Multiplexing sensitivity of each component was found to be less than 25 copies of the target. Mixed infections could be easily detected using the different channels of the PCR instrument. Specificity of the assay was assessed by a cross-reactivity panel. Results from the validation panels yielded 100% concordance with the expected resistance patterns.
Conclusions: The 3base™ technique expands the detection capacity of multiplex-PCR for some target genes (CTX-M) to detect various subtypes within the target by affecting the DNA sequence homology. Also, novel variants or new resistant markers can readily be incorporated into existing assays easily given the properties of the 3base™ converted DNA sequences, thus improving the throughput of such assays. The optimized assay provides a sensitive and specific alternative for the detection of ESBL and CPO and can be carried out in less than 3 hours with minimal hands-on time for laboratory technicians.

P4

Rapid, sensitive and automated detection of 12 bacterial and viral causes of sexually transmitted infections with EasyScreen™ STI assay

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Background: Sexually transmitted infections (STIs) have a significant impact on sexual and reproductive health, with the World Health Organization reporting more than 1 million STIs being acquired on a daily basis. The four most commonly reported STIs are chlamydia, gonorrhoea, syphilis, and trichomoniasis. Other pathogens of STI include *Mycoplasma spp.*, *Ureaplasma spp.*, and herpes simplex virus. The new 3base STI assay is a simple and rapid molecular method that utilizes 3base™ technology to modify the 4 usual DNA bases into only 3 bases (A, T, G) via a novel, patented bisulphite conversion step. Nucleic acids are converted to a 3base™ form during the DNA isolation in order to yield better multiplexed PCR performance.
Objectives: To develop a novel 3base™ real-time PCR (RT-PCR) assay for detection of the presence of 12 most significant and commonly encountered STIs in less than 4 hours from primary patient material.
Materials and methods: The assay sensitivity was determined using quantified genomic DNA controls from Vircell and assay performance assessed by using reference material from ATCC, Zeptometrix, and QCMD. The clinical performance of the assay was assessed by using over 800 clinical isolates obtained from St. Vincent's Hospital. DNA extraction and PCR setup were performed on a Hamilton Nimbus platform, especially modified for Genetic Signatures, PCR amplification and detection on a CFX384 Touch™ (Bio-Rad) real-time PCR instrument.
Results: The new 3base™ STI assay was found to be highly specific with no cross reactivity observed. There was 100% concordance with all targets including CT/NG and HSVs compared to conventional molecular methods used at the hospital. 25.1% of mixed infections were easily detected using the new assay.
Conclusions: The new 3base™ STI assay provides a sensitive and specific alternative for the detection of STI pathogens. The workflow from sample processing to results requires less than 4 hours with minimal hands on time. This is particularly advantageous not just for high-throughput laboratories but also in improving patient diagnosis and management.

P5

SLCO1B1 521 T>C polymorphism distribution in the Austrian population performed with two real-time PCR-assays: a clinical implementation study

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Background: Statins are widespread lipid-lowering agents prescribed for the prevention of cardiovascular disease and mortality. Statin-induced myopathy is reported to be significantly associated with the *SCLO1B1* c.521T > C polymorphism. Detailed epidemiologic data about *SLCO1B1* c.521T > C testing among Austrian individuals are still lacking.
Objectives: To assess the genotype and allele frequencies of the *SLCO1B1* c.521T > C variant in Austria and to evaluate the clinical performance of two commercially available real-time (RT)-PCR assays.
Materials and methods: Samples obtained from 181 healthy Austrian subjects (test set) were genotyped for the *SLCO1B1* c.521T > C polymorphism with the recently launched *SLCO1B1* c.521T > C RealFast™ Assay (ViennaLab Diagnostics GmbH, Vienna, Austria). Of these, 88 DNA samples (training set) were used to compare the genotyping results with the BioPro *SLCO1B1* Genotyping RT-PCR Kit (Bio Products, Stockerau, Austria). Cohen's Kappa (κ) was used to calculate the agreement between the two RT-PCR assays.
Results: All in all, 10 (5.5%) and 44 (24.3%) out of 181 individuals were *SLCO1B1* c.521T > C C/C-homo- and C/T-heterozygotes, *SLCO1B1* c.521T > C genotypes indicative of high and increased risk of statin-induced myopathies. A total of 127/181 (70.2%) could be identified as T/T homocygotes, the *SLCO1B1* genotype associated with normal myopathy risk. Frequencies of c.521C- and c.521T-alleles were 17.7 and 82.3%, respectively. In all genotype groups, concordance between the two RT-PCR assays was 100%. Cohen's κ demonstrated perfect observed agreement ($\kappa = 1$, $P < 0.001$).

Conclusions: The genetic predisposition of elevated statin-induced myopathy risk in Austrian subjects is frequent. Both RT-PCRs evaluated here are reliable and robust diagnostic tools for pharmacogenetic *SLCO1B1* c.521T > C testing in clinical routine.

P6

Arterial stiffness in a black South African cohort infected with the human immunodeficiency virus (HIV)

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Background: Carotid-femoral pulse wave velocity (PWV) is a surrogate marker of atherosclerosis and predictive of cardiovascular events, morbidity, and mortality. HIV is associated with hypertension, dyslipidemia, and endothelial dysfunction resulting in increased stiffness. Studies comparing arterial stiffness in HIV-infected to HIV-free individuals are scarce, especially in South Africa, one of those countries most-affected by HIV, and with a high prevalence of hypertension.
Objectives: To compare arterial stiffness between HIV-infected and HIV-free black individuals.
Materials and methods: HIV-infected participants (n = 139, 72% infected >10 years) were age, sex, and locality matched to HIV-free controls. Carotid-femoral PWV and central systolic blood pressure (BP) were measured with the SphygmoCor® XCEL device, while brachial BP was measured with the OMRON M6. Of the HIV-infected participants, 73% were on antiretroviral treatment.
Results: All BP measurements were lower in those infected and the prevalence of hypertension (41 vs 29%, $p = 0.032$) was higher in the HIV-free controls. PWV did not differ between the groups and neither locality nor 10-year duration of infection influenced PWV. Among the >60-year-old age group, PWV tended to be lower in the infected participants ($p = 0.062$). A trend of increasing PWV with increasing age ($p = 0.011$) was seen in the HIV-free controls only. PWV was lower ($p = 0.046$) in normotensive HIV infected participants compared to normotensive controls, but did not differ among hypertensive participants.
Conclusions: In this HIV-infected African cohort, increased stiffness was not detected. The higher prevalence of hypertension in the HIV-free group may, at least in part, have influenced the results.

P7

Evaluation of two new molecular diagnostic assays to facilitate the analysis of gastrointestinal infections

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Background: Clinical diagnostic laboratories are facing a growing demand to streamline internal processes and to provide results at reduced turnaround times without exceeding budgets. Emergency requests and peaks in sample numbers further burdens the operational procedures. Today's standard molecular diagnostic tests are often time consuming or provide fast results at high costs. Acceleration of diagnostic processes could optimize equipment usage and improve organizational flexibility.
Objectives: To evaluate two fast real time PCR-based assays for the qualitative detection of *C. difficile*- and Norovirus-specific nucleic acids. To assess the overall time saving potential of these kits.
Materials and methods: 193 and 201 clinical stool specimens were prospectively analyzed with the Anchor Norovirus PCR Kit and the Anchor C. difficile PCR Kit, respectively. Nucleic acids were purified using the NucliSENS® easyMag® (bioMérieux). All results were aligned with the routine diagnostic outcome based on the RealStar® Norovirus RT-PCR Kit 2.0 (altona Diagnostics) and the RealStar® Clostridium difficile PCR Kit 1.0 (altona Diagnostics). All assays were processed on the Rotorgene Q (QIAGEN). The positive and negative percent agreements (PPA / NPA) between the products were determined to assess the clinical performance of the Anchor PCR Kits.
Results: The Anchor Norovirus PCR Kit showed a PPA of 100% and a NPA of 96.0% when compared to the RealStar® Norovirus RT-PCR Kit. Three out of five discrepant results initially negative with the altona Kit gave positive results after retesting, while two results remained negative. Comparison of *C. difficile* PCR assays generated three discrepant samples. All of them were tested positive with the Anchor C. difficile PCR Kit but negative using the comparator assay. The Anchor C. difficile PCR Kit showed a PPA of 100% and a NPA of 97.8%. Run times of the Anchor Norovirus PCR Kit and the Anchor C. difficile PCR Kit were 45 and 39 minutes, respectively.
Conclusions: Both the Anchor Norovirus PCR Kit and the Anchor C. difficile PCR Kit showed a highly competitive clinical performance and a massively reduced cycling time providing economy of time of more than 60%.

P8

Comparison of assays for determination of HCV genotypes and recombinants using different molecular techniques

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Background: According to the most recent EASL recommendations on the management of hepatitis C virus (HCV) infection, HCV genotypes (GT) and GT 1 subtypes (1a or 1b) should be assessed prior to treatment initiation, as this will impact the choice and duration of direct-antiviral treatment regimens. Currently, HCV is classified into 7 different GTs and 67 subtypes. Additionally, several natural intra- and intergenotypic recombinants of HCV have been identified. Among these, the so-called "St Petersburg variant", a genotype 2k/1b recombinant, is the most prevalent worldwide.
Objectives: To compare results obtained by assays using different molecular techniques for determination of HCV GTs 1 and 2 as well as recombinant forms between these two genotypes.
Methods: In this study, 279 samples derived from patients with chronic HCV infection were investigated. HCV GTs and subtypes were determined by the reverse hybridization-based VERSANT® HCV Genotype 2.0 Assay (LiPA; Siemens Healthcare) that targets the 5´UTR and HCV core regions, and the real-time PCR based cobas® HCV GT (cobas; Roche Molecular Systems) assay that targets the 5´UTR, core, and NS5B regions. Assay results were compared to direct sequencing of the HCV core, NS2/NS3 junction, NS3, NS5A, and the NS5B regions as the reference standard.
Results: In total, 53 patients had subtype 1b, 177 patients had GT2, and 48 patients had GT 2/1 recombinants according to direct sequencing (2k/1b, n = 46; 2b/1a, n = 1; 2a/1b, n = 1). Finally, one patient had a mixed 1b+2b infection. All 53 samples with subtype 1b were correctly subtyped by LiPA, and 51/53 samples were subtyped with the cobas assay, respectively (two samples yielded GT1 results only). Among GT2 samples, 177/177 and 174/177 samples were correctly reported as GT2 by the LiPA and cobas assay, respectively (three samples yielded invalid results with the cobas assay). LiPA reported all 48 GT2/1 recombinants as GT2, whereas cobas reported 43 as GT1b/2 (which cannot rule out double infection) and five samples were reported as GT2 only. One sample was identified as mixed GT1+GT2 infection when results obtained by both assays were taken into consideration.
Conclusions: When analyzing HCV patient samples containing strains without recombination, analysis of the 5´UTR and core regions is sufficient, while identification of HCV recombinant forms requires inclusion of an additional region close to the 3´ end of the HCV genome.

P9

Epidemiology of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and urogenital mycoplasma infections in central Slovenia

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Background: Infections with *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and urogenital mycoplasmas (GM) have been recognized as sexually transmitted infections (STI). Therefore, knowledge on CT/NG and GM epidemiology is important to better understand the burden of these pathogens in the population.
Objectives: To obtain data on CT/NG and GM epidemiology in the Slovenian population and to evaluate the performance of two real-time PCR assays for detection of CT/NG in clinical samples.
Materials and methods: Urogenital, ocular, and throat swabs collected in 2SP media from symptomatic patients in 2017 were enrolled in this study. The cobas® 4800 System (Roche) was used for CT/NG detection. Simultaneously, identical samples were used for automated nucleic acid (NA) extraction using MagNA Pure Compact (Roche) and further analyzed by multiplex PCR with the Allplex™ STI Essential assay (Seegene).
Results: Overall, 1870 tests for CT, 1168 for NG, and 1527 for GM were performed. The most common sexually transmitted pathogen was *Ureaplasma parvum* (Up) with 22.9% (350/1527) followed by CT with 12.5% (233/1870), *Ureaplasma urealyticum* (Uu) with 9.2% (139/1527), *Mycoplasma hominis* (Mh) with 5.9% (90/1527), NG with 5.2% (61/1168), and *Mycoplasma genitalium* (Mg) with 1.8% (27/1527). Coinfections were found in 143 patients. The most common combination was Up+Mh (33/143) followed by Up+CT (23/143), Uu+Up (13/143), Uu+CT (10/143), and Uu+Up+Mh (10/143). Other combinations were detected rarely.
Conclusions: In 2017, the most common STI among symptomatic patients in Slovenia was Up followed by CT. The most common mixed infections were Up+Mh and Up+Ct.

P10

Relationship between CD4 count and creatinine/albumin ratio in HIV-positive patients

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Background: Renal dysfunction is relatively common among HIV-infected persons. It is, however, not clear whether renal dysfunction is the consequence of HIV infection or antiretroviral therapy or both.
Objectives: To assess renal function in HIV-negative and HIV-positive persons.
Materials and methods: Seventy-two participants (41 females and 31 males) aged 21 to 51 years were recruited for the study. Body mass was measured, blood and spot urine samples were collected and analyzed for creatinine, albumin/creatinine ratio, and CD4 count.
Results: There was no difference in mean age and body mass between male and female subjects. CD4 counts were higher in females compared to males (640 ± 86 vs. 434 ± 43; $p = 0.06$). Serum creatinine levels were significantly lower in HIV-positive females compared to males (70.0 ± 2.0 µmol/l vs. 75.4 ± 2.7 µmol/l, $p < 0.001$), while there was no difference found in urinary creatinine levels. While none of the HIV-negative subjects showed a raised albumin/creatinine ratio, 14% of the HIV-positive population had albumin/creatinine ratios >3.0 mg/mmol. There was a positive correlation between CD4 count and albumin/creatinine ratio in female patients on antiretroviral therapy ($r = 0.91$, $p < 0.01$), while male participants in the same category showed no relationship between the two variables.
Conclusions: These preliminary results suggest that there is a relationship between CD4 count and albumin/creatinine ratio. Renal function impairment may be associated with HIV-infection in patients in Mthatha, South Africa.

P11

HIV/AIDS and atherosclerotic cardiovascular disease

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Background: Worldwide, cardiovascular disease and infectious diseases (mainly HIV) are among the top ten causes of death. Currently, over 35 million people live with HIV. Due to advances in antiretroviral therapy regimes, life expectancy has risen significantly. Therefore, non-communicable diseases have come into focus in research as well as the management of HIV-positive individuals.
Objectives: To provide an overview of the current literature on HIV/AIDS and antiretroviral therapy and their effect on the cardiovascular system.
Materials and methods: Over 500 articles, which were gained from Pubmed and Web of Sciences searches as well as appropriate citations, were reviewed based on their abstracts and included if relevant in this review.
Results: While traditional cardiovascular risk factors (especially smoking) has been more prevalent in the HIV population, HIV and antiretroviral therapy themselves are also considered as independent risk factors. Emerging chronic infection and inflammatory state need to be taken into account as well. Enhanced changes in vascular function and structure have been shown in many studies, leading to the assumption of an accelerated progression of atherosclerotic disease in people with HIV. However, while there is a great number of studies from high-income countries, studies from the most affected region of the world, Sub-Saharan Africa, are scarce. A number of questions regarding possible cultural, ethnical, and geographical differences thus remain unanswered.
Conclusion: The pathogenesis of atherosclerotic cardiovascular disease in the HIV population is multifactorial. Effects of HIV and antiretroviral therapy cannot always be distinguished. Future studies should aim at developing appropriate guidelines for monitoring and management of cardiovascular disease. Ideally, these guidelines should also consider possible ethnical and geographical differences.

P12

Antibiotic resistance in *Streptococcus pneumoniae* isolates: comparison of patients with cystic fibrosis and patients with airway disorders presenting at the central emergency admission

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Background: *Streptococcus (S.) pneumoniae* is a relevant pathogen involved in various diseases such as otitis media, pneumonia, and meningitis. Prevalence of nasopharyngeal colonization in the healthy population is 10-50%. Colonization rates in cystic fibrosis (CF) patients is unknown, but there is an increased pathogenic potential of *S. pneumoniae* in patients with CF and increased antibiotic resistance.
Objectives: To compare antibiotic resistance profiles in *S. pneumoniae* obtained from patients with cystic fibrosis and those obtained from patients with airway disorders presenting at the central emergency admission.
Material and methods: Microbiological databases were reviewed on patients with nasopharyngeal colonization with *S. pneumoniae* in 2016 and 2017. Patients with CF and those with airway disorders presenting at the central emergency admission of the University Hospital Graz were studied.
Results: Colonization with *S. pneumoniae* was found in nasopharyngeal swabs of 27/118 (22.9%) patients with CF. Corresponding data for non-CF patients were 21/1130 (1.9%). Of 27 isolates obtained from CF patients, 7 (25.9%) were found to be non-susceptible, but not fully resistant (intermediate) against penicillin. Full resistance against tetracycline, erythromycin, and clindamycin was detected in 3 of 27 (11.1%) isolates. Of 21 isolates obtained from non-CF patients, 4 (19.0%) were found to be non-susceptible, with one of them fully resistant against penicillin. Full resistance against tetracycline, erythromycin, and/or clindamycin was detected in 5 of 21 (23.8%) isolates.
Conclusions: In the present study, a significantly higher prevalence of *S. pneumoniae* in isolates of CF patients compared to non-CF patients was observed. There was an increased number of antibiotic resistance in *S. pneumoniae* isolates in patients with CF. However, full resistance was found more frequently in patients with airway disorders presenting at the central emergency admission.

P13

Sequence variability in the HCV core gene correlated with clinicopathological features

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Background: The hepatitis C virus (HCV) core protein is associated with cellular transformation. Transgenic mice containing the HCV core gene develop steatosis and liver cancer. Several studies revealed that HCV-core sequences in patients with hepatocellular carcinoma (HCC) differ from those of patients with early-stage liver disease. They identified seven mutations in the viral core gene significantly associated with increased HCC risk. Understanding the relationship between viral mutations and liver disease may suggest new targets for therapeutic intervention.
Objectives: To determine the HCV core gene sequence diversity in plasma and liver biopsies of chronically infected, treatment naive patients and to correlate HCV core gene mutations with basal viremia, HCV subtype, and histopathological parameters of liver disease.
Materials and methods: Paired plasma samples and liver biopsies from 85 HCV-infected, treatment-naive patients were studied. After HCV RNA extraction, viral RNAs were transcribed to cDNA and the entire HCV core gene was amplified. Amplification products were sequenced with the Big Dye termination v1.1 method on an ABI 310 sequencer (Applied Biosystems). HCV core sequences obtained were compared to reference HCV genotypes from GenBank.
Results: The distribution of HCV subtypes in the study group was as follows: 28 patients with subtype 1a, 30 patients with subtype 1b, and 27 patients with subtype 3a. Identical HCV core region sequences were found in paired samples of 76/85 patients. The most frequent and statistically significant substituted amino acids (aa) were R70Q/H, T75A, and M91L/C ($p = 2.8x10^{-5}$, $p = 1.9x10^{-12}$, $p = 1.7x10^{-5}$, respectively). HCV subtype 1b and basal viremia were significantly correlated with presence of R70Q/H, T75A, and M91L/C substitutions. Hepatitis activity grade and liver fibrosis were associated with presence of R70Q and M91L/C substitutions. Distribution of the HCV core aa substitutions was found to be subtype-specific and demonstrated conserved blocks (regions with no substitutions) and hot spots with frequent substitutions.
Conclusions: Specific amino acid substitutions in the core region may be correlated with progression of liver disease and could serve as a prognostic marker for disease outcome.

P14

Demographic and cardio-metabolic factors in a HIV-infected South African cohort over 10 years

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Background: South Africa is burdened by a high prevalence of human immunodeficiency virus (HIV) with approximately 7.1 million people living with HIV. Increasing evidence indicates that cardio-metabolic and renal diseases are becoming common in HIV-infected patients.

However, longitudinal studies are still lacking in order to understand the connection between HIV infection and cardiovascular disease (CVD) risk in in people living with HIV, especially in Africa.
Objective: To profile demographic and cardio-metabolic factors as well as measures of renal function of an HIV-infected African population over 10 years.
Materials and methods: This study is embedded in the Prospective Urban and Rural Epidemiology (PURE) study. We profiled a cohort of 320 HIV-infected and 320 HIV-uninfected participants from the North West Province, South Africa. The participants were matched for age, gender, locality, and body mass index. By using standardized procedures, demographic, lifestyle, cardio-metabolic, and renal function variables of these participants were studied in 2005, 2010, and 2015.
Results: With 89.6% of the HIV-infected participants using antiretroviral therapy in 2015, a decrease in mortality from 24% (2005-2010) to zero (2010-2015) was observed. Although no differences in blood pressure and body composition between the groups were seen over 10 years, HIV-infected participants showed greater increases in total cholesterol, high density-lipoprotein cholesterol, and low density-lipoprotein cholesterol compared to HIV-uninfected participants (all $p < 0.001$). This was accompanied by greater increases in C-reactive protein (CRP) ($p = 0.047$) and gamma glutamyltransference (GGT) ($p < 0.001$). With regard to renal function, no significant difference was observed in urinary albumin excretion between these groups, but HIV-infected participants showed a larger decrease in estimated glomerular filtration rate ($p = 0.003$). These results were confirmed, except for CRP, after adjusting for age and sex (all $p = 0.005$).
Conclusions: South Africans living with HIV for 10 years showed longitudinal increase in lipid profile and as well as greater increases in inflammation, GGT and decrease in renal function over time. These markers are associated with increased CVD risk and advocate for regular screening/monitoring of CVD risk factors in the HIV-infected population.

P15

Therapeutic management of HIV positive patients by a new real time PCR device for viral DNA quantification

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Background: Quantification of HIV DNA load in peripheral blood mononuclear cells is considered an important and useful tool for therapeutic management of patients with HIV infection.
Objectives: To develop a new device, REALQUALITY RQ-HIV DNA, based on the real time PCR technology, for the quantification of proviral HIV-1 DNA.
Materials and methods: Specific primers and probes were designed for the LTR region of the HIV genome by analyzing multiple alignment of 3500 HIV sequences, registered in the reference database (http://www.hiv.lanl.gov). Analytical specificity was evaluated both *in silico* and *in vitro* with spiked samples containing specific LTR sequences of several HIV subtypes. Analytical sensitivity was calculated by using serial dilutions of a plasmid containing the HIV-1 target sequence (LTR region of HIV-1 subtype B). In addition, different concentrations of extracted DNA obtained from the cellular line 8e5 (NIH AIDS reagents program) were investigated. Diagnostic performance was evaluated on a total of 40 clinical samples and on 2 External Quality Assessment (EQA) QCMD panels. An in house assay, targeting the *pol* gene, was used as reference method.
Results: Primers and probes were able to correctly identify HIV 1, group M, subtypes A, B, C, F, G, AE, AG, as demonstrated by results of both *in silico* and *in vitro* analyses. Analytical sensitivity was defined as 10 copies/reaction on 10^6 cells. Preliminary diagnostic performance showed a high analytical sensitivity and specificity. Good correlation of the quantification data was observed between the REALQUALITY RQ-HIV DNA kit and the reference method.
Conclusions: REALQUALITY RQ-HIV DNA showed a high analytical specificity identifying the major viral subtypes of HIV-1 group M. Analytical and clinical sensitivities of the new kit satisfied requirements necessary for monitoring the efficacy of treatment. REALQUALITY RQ-HIV DNA may be useful for therapeutic management of HIV-infected patients.

P16

Cervical cancer screening and triage of uncertain cytological outcomes in ULSS 5 – Ovest Vicentino

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Background: The cervical cancer screening algorithm implemented in ULSS 5 – Ovest Vicentino uses HPV DNA testing for triage of patients with ASCUS, L-SIL, and AGC Pap Test results as well as patients with two consecutive rounds of inadequate pap smears.
Objectives: To evaluate triage outcome with regard to epidemiological data and impact on the management of patients.

Materials and methods: From May 2012 to May 2013, 6730 cervical smears were analyzed in the context of the local screening program and classified according to Bethesda 2001 criteria. Molecular analysis was then performed on 470 samples from patients with uncertain cytological indication for colposcopy referral (triage population) and on 13 samples from patients, already referred for colposcopy, that could have benefited from HPV genotyping from the prognostic point of view. Thus, a total of 483 samples were tested with AMPLIQUALITY HPV-TYPE EXPRESS (AB ANALITICA), a HPV genotyping device based on GP5+/GP6+ PCR amplification and reverse line blot.
Results: 224 samples resulted negative for HPV, whereas 259 were positive. All positive patients were referred for colposcopy and 171 were subjected to biopsy resulting in 27 cases of CIN 2-3, and 3 cases of adenocarcinoma. Of 224 patients with negative results, 13 were referred for colposcopy due to reconsideration of their cytological status. Six of them were subjected to biopsy resulting in low grade histological outcome. A single high risk (HR) HPV type was found in 74% and a single low risk (LR) HPV type in 19% of patients with positive results. In the remaining 7% of patients, co-infections were detected. HPV 16 and 42 were the most common HR and LR types respectively. Half of CIN2+ results were attributable to infection caused by HPV type 16.
Conclusions: Nearly half of the triage population included in this study resulted negative for HPV according to molecular analysis and was thus returned to routine recall positively affecting second level patient management from both the economical and the organizational point of view.

P17

Comparison of Cepheid GeneXpert® and Roche LIAT© assays for the detection of influenza A, influenza B, and RSV

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Background: Influenza viruses spread rapidly, cause respiratory diseases in all age groups, and are associated with considerable morbidity and mortality worldwide. Rapid laboratory confirmation of suspected influenza virus infection facilitates treatment decisions and leads to an earlier administration of influenza virus-specific antivirals.
Objectives: To compare Cepheid GeneExpert® and Roche LIAT© assays for rapid PCR-based detection of influenza A, influenza B, and RSV.
Materials and methods: In this single-center study, 638 prospectively collected samples (throat and nasal swabs) of patients with symptoms compatible with influenza-like-illness or acute respiratory infections were tested for the presence of both influenza and RSV. For this purpose, two LIAT© systems were installed, one at the Department of Emergency Medicine and one at the Department of Laboratory Medicine. The study was divided into two arms: 338 samples of patients visiting the Emergency Department were analyzed by the LIAT© system directly at the Emergency Department and additionally by the GeneExpert® system at the Department of Laboratory Medicine. In the second arm, 300 random samples of hospitalized and/or outpatients of the Medical University of Vienna were analyzed with the LIAT© system and the GeneExpert® system both located at the Department of Laboratory Medicine. Samples were tested twice with the GeneExpert® system using both the novel Xpert® Xpress Flu/RSV assay as well as the Xpert® Flu assay. Sample analysis was performed within 24hours after collection.
Results: 250 of 638 samples were positive for either influenza A, H1N1, influenza B, or RSV. There was 90.0% overall agreement between the Xpert® Flu assay and the LIAT© assay. 98.3% overall agreement was found between the Xpert® Xpress Flu/RSV assay and the LIAT© assay. The sensitivity of the LIAT© Influenza A assay was 98.1%, 93,7% for the LIAT© Influenza B assay, and 100% for the LIAT© RSV assay.
Conclusions: The LIAT® system represents a robust and highly sensitive POCT device for the rapid PCR-based detection of influenza A, influenza B, and RSV.

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Evaluation of three molecular assays for rapid testing of seasonal influenza in patients requiring hospitalization

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Background: Patients with seasonal influenza pose an infection control challenge. Nosocomial spread of influenza endangers high-risk patients. Isolation or separation of influenza patients is highly recommended. Rapid diagnostic test results are thus crucial. Previously used rapid antigen tests have a very low sensitivity (25-71%), while PCR results lack timely delivery.
Objectives: To overcome diagnostic weakness by evaluation of three rapid molecular tests suitable for near-patient testing.
Materials and methods: 271 patients with influenza-like illness presenting at the emergency department of the Graz University Hospital during the season 2017/2018 with at least one risk factor for complications were studied. Specimens were obtained with Copan UTM® 3mL-liquid transport medium. Specimens were tested for influenza A and B RNA using the Influenza A&B Alere™i (Alere™), GeneXpert Xpert®Xpress

Flu/RSV (Cepheid), and the Cobas® Liat® (Roche) systems. Results were compared to those obtained by Influenza A/B r-gene® (Biomerieux) real-time PCR as reference method.

Results: Of 271 specimens, six were excluded due to a lacking cell signal control with the reference assay. The reference assay gave 41 positive results for influenza A and 80 for influenza B. Cobas Liat yielded invalid results in 32 cases (all of them positive by reference PCR, one for influenza A and 31 for influenza B) that were excluded from further analyses. Sensitivities and specificities of three rapid molecular tests are shown in Table 1.

Conclusions: During the influenza season 2017/2018 with a very high proportion of circulating influenza B, overall specificity of the three rapid molecular tests was very high (>95%). The highest overall sensitivity was reached with GeneXpert (97.5%). The lowest overall sensitivity showed Alere i (89.3%). This effect was mainly due to a very low sensitivity for influenza A (78.0%). Liat showed a relatively poor performance for influenza B due to 32 invalid test results.

Table 1: Sensitivities and specificities of three rapid molecular tests.

	Alere i (n = 265)	Liat (n = 233)	GeneXpert (n = 265)
Results for influenza A and B			
Sensitivity	89.3%	95.5%	97.5%
Specificity	99.3%	97.9%	98.6%
Results for Influenza A			
Sensitivity	78.0%	100%	97.6%
Specificity	99.6%	98.4%	99.6%
Results for Influenza B			
Sensitivity	96.3%	93.9%	97.5%
Specificity	100%	100%	99.5%

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