AUTHOR INDEX OF ABSTRACTS

Name:	Prefix:	Firstname:	Abstract number:
Buettner		Reinhard	**81**
Experiences from the Network of Genomic Medicine (NGM)			
Castriciano		Santina	**31**
MSwab Allows Viral Detection with Direct-Rapid Nucleic Acids Amplification and Culture Assays			
Cernochová		Michaela	**5**
Comparison of six commercial kits for pathogen DNA extraction from whole blood			
Chmielarczyk		Agnieszka	**27**
Molecular epidemiology and drug resistance of Acinetobacter baumannii isolated from ICU in hospitals of southern Poland.			
Cnops		Lieselotte	**48**
Evaluation of a rapid ebola virus triage test on the idylla™ system			
Dekter		Hinke	**14**
Single cell MALDI-TOF based typing of Serratia marsescens strains obtained from hospitalized patients.			
Del Favero		Jurgen	**66**
Multicenter performance evaluation of BRCA MASTR™, CFTR MASTR™ and FMF MASTR™ on Roche 454™ and Illumina MiSeq® massively parallel sequencing platforms			
Deras		Ina	**78**
A Next-Generation Sequencing Method for Identifying Somatic Variants from FFPE Solid Tumor Samples			
Descheemaeker		Patrick	**28**
MolecularDiagnostics.be 2014 external quality control scheme on the molecular detection of Mycoplasma genitalium and Trichomonas vaginalis			
Dewit		Emmie	**94**
Ebola virus outbreak: working in a field diagnostic laboratory in Liberia, 2014-2015			
Di Lorenzo		Caterina	**60**
Proficiency testing programmes as a tool to assess and improve diagnostic methods for infectious diseases.			
Eboigbodin		Kevin	**41**
Reverse Transcription Strand Invasion Based Amplification (RT-SIBA) Method for Rapid Detection of Human Rhinoviruses			
Ehret		Robert	**3**
Hologic Aptima HIV-1 Quant Dx Assay Compared to Abbott RealTime in B-/Non-B-Subtypes in daily routine testing			
Ehret		Robert	**42**
The newly developed Cepheid Xpert® HIV-1 VL compared to the established Abbott RealTime HIV-1 viral load measurement			

Name:	Prefix:	Firstname:	Abstract number:
Eloit		Marc	**85**
The diagnostic of infectious diseases by Whole-Genome Next Generation Sequencing			
Engeland	van	Manon	**95**
Molecular markers for detection of colorectal cancer			
Enroth		Helena	**12**
NGS pilot study of E coli ESBL from patients with suspected sepsis			
Estampes		Audrey	**26**
CE-IVD validation of a new molecular diagnostics sample-to-result solution in combination with transplant monitoring RT-qPCR assays			
Galán			**70**
Molecular identification of species belonging to Aspergillus section Nigri isolated in clinical samples (FUNGAE Study Group)			
Giambra			**30**
Evaluation of Copan FecalSwab for detection of enteric pathogens with Nucleic Acid Amplification Techniques			
Gizzie		Nina	**71**
Recovery of Genomic DNA and isolation of 16S Ribosomal DNA from Sigma Transwab® system with viability acceptance in accordance with CLSI M40-A2 standard			
Goh		Michelle	**25**
Development and evaluation of a fluorescent pentaplex assay for microsatellite instability in colorectal cancer			
Hagen		Annegret	**59**
Lyophilized real-time PCR kits from Fast-track Diagnostics: faster and even easier			
Hays			**8**
Multi-omics diagnostics for tailoring treatment in respiratory tract infections and sepsis.			
Hirvonen		Tommi	**58**
Improvement of diagnostic method validation processes in a Finnish hospital laboratory using lean methods and a software solution			
Hodinka		Richard	**87**
The Miniaturization of Molecular Technologies for Infectious Disease Testing			
Holden		Matthew	**89**
Whole genome sequencing as a tool for micro-epidemiological investigations, what have we learnt?			
Huijsmans			**13**
Cervical cancer screening in the netherlands: determination of hpv Prevalence using three different systems.			
Ingelman-Sundberg		Magnus	**83**
Pharmacogenomic and epigenomic biomarkers for predicting interindividual differences in drug metabolism and response			

Molecular Detection of Mycobacterium tuberculosis: 5 Years of External Quality Assessment

Name:	Prefix:	Firstname:	Abstract number:
Micalessi		Isabel	**36**
Evaluation of the idylla braf mutation assay performance using the Novel fully-automated idylla system on formalin-fixed parafin Embedded melanoma samples			
Micalessi		Isabel	**37**
Evaluation of the idylla braf mutation assay performance using the Novel fully-automated idylla system on formalin-fixed parafin Embedded colorectal cancer samples			
Mokany		Elisa	**47**
Highly multiplexed assay for detection of antimicrobial resistance using PASS MNAzyme qPCR: Mycoplasma genitalium and macrolide resistance			
Molenkamp		Richard	**4**
Pacbio sequencing of the genetic diversity of the hepatitis c virus Envelope region: from early acute to chronic infection			
Mulder		Sije	**56**
Validation of the Cepheid Xpert MRSA Gen3 cassette.			
Nijhuis		Roel	**50**
Comparison of diagnostic and bioinformatics real-time PCR assays for detection of human astroviruses			
Noguera-Julian		Marc	**82**
NGS data analysis, quality aspects for diagnostics			
Oostra		Monique	**15**
Development of 2 multiplex real-time PCR assays for the detection of the Celiac disease related Humaan Leukocyte Antigens DQ2.5, DQ2.2 en DQ8			
Op den Buijs		Ingrid	**2**
Evaluation of a Rapid Molecular Method to Detect Mycobacterium tuberculosis using the BD-MAX system in a European Multicenter Study.			
Orelio		Claudia	**11**
Rapid antimicrobial susceptibility testing in bacteremic patients with Raman spectroscopy			
Orelio		Claudia	**21**
Molecular fingerprint of mussel enterobiome is associated with the geographical location			
Padalko		Elizaveta	**43**
Human papillomavirus and Chlamydia trachomatis testing on same specimen			
Pelt-Verkuil	van		**10**
Development of an internal process control (IPC) and analysis of PCR-artefacts			
Poelman		Randy	**93**

Point-of-impact testing in the emergency department: rapid Diagnostics for respiratory viral infections

Name:	Prefix:	Firstname:	Abstract number:
Radersma		Joyce	**61**
Validation and implementation of Next Generation Sequencing in pathology diagnostics			
Ramalingam		Sandeep	**65**
Change in viral shedding associated with hypertonic saline nasal irrigation and gargling: ELVIS, a pilot randomised controlled trial			
Raymaekers			**63**
Technical validation of three commercial real-time PCR kits for the diagnosis of neuroborreliosis in cerebrospinal fluid on three different real-time PCR platforms.			
Raymaekers			**67**
Five years of experience with ISO 15189 accreditation in Belgian molecular diagnostics laboratories. Presented on behalf of MolecularDiagnostics.be			
Roepman		Paul	**57**
Differentiation between second primary lung cancers and metastatic disease using next-generation sequencing			
Rossen		John	**9**
Transmission of high risk K. pneumoniae clones in health care networks largely challenges the current infection prevention and control system			
RUPPE		Etienne	**55**
Clinical metagenomics: a new paradigm for the diagnosis of infectious diseases?			
Schaik	van	Ron	**86**
DNA diagnostics for guiding drug therapy: latest developments at the European level			
Schee	van der	Marc	**90**
Analysis of volatile metabolites for disease diagnosis, phenotyping and prognosis.			
Shin			**32**
Microfluidic-based non-enzymatic glycation enhances the cross-linking of human scleral tissue compared to conventional soaking			
Smoldovskaya		Olga	**1**
Hydrogel microarray platform for in vitro allergy diagnostics			
SQUASSINA			**62**
Copan eNAT Medium Stabilize Nucleic Acids for the Detection of Viruses with the xTAG Respiratory Virus Panel			
Tabb		Michelle	**33**
Simplexa™ C. difficile Direct—A Sample-to-Answer Solution for Labs Combining Real-Time PCR Detection with Simplified Workflow			

Terlouw-Klein Marijke **23**
Comparison of the Performance and Workflow of the Aptima HIV-1 Quant Dx Assay and the COBAS® Ampliprep/COBAS® Taqman HIV-1 Test for Quantitation of HIV-1 RNA.

Name:	Prefix:	Firstname:	Abstract number:
Trijp	van	Maria	**44**
The relationship between Ct-value in Clostridium difficile positive feces samples and fecal consistency			
Trijp	van	Maria	**45**
The relationship between detected Ct-value in Salmonella enterica positive feces samples and fecal consistency			
Vaeyens		Freya	**7**
A duplex real-time TaqMan PCR assay for the rapid and reliable detection of allele HLA-B*27 in the Caucasian population.			
Vanlaere		Elke	**54**
Implementation of the BD MAX^{TM} system for detection of Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis using E-swab^{TM}			
Vanmassenhove		Ben	**84**
Validation and clinical performance of an in-house JAK2V617F mutation detection assay on the Rotor Gene Q			
Willems		Philippe	**22**
Comparing the technical performance of two real-time PCR kits for the detection of Trichomonas vaginalis in the laboratory			
Worthey		Elisabeth	**92**
'What clinically useful information can whole genome sequencing of rare or complex disease suffering or of healthy individuals provide?'			
Wujcicka		Wioletta	**18**
GC heterozygotic status at IL6 -174 G>C SNP and increased risk of congenital infection with Toxoplasma gondii			
Wujcicka		Wioletta	**19**
Polymorphisms of genes encoding Toll-like receptors and cytokines and development of congenital cytomegaly			
Wurdinger		Thomas	**80**
RNA-seq of tumor-educated platelets enables blood-based pan-cancer, multiclass and molecular pathway cancer diagnostics			

KEYNOTE ABSTRACTS BY ORDER OF SESSION NUMBER

Keynote abstracts

Abstract number: 85
(Session 1A)

The diagnostic of infectious diseases by Whole-Genome Next Generation Sequencing
Prof. M Eloit
Institut Pasteur, Paris, France

The laboratory diagnostic of bacterial, viral and fungal diseases has long been dominated by culture, a method that does not necessitate any diagnostic hypothesis, but is limited by the non-cultivability of many pathogen species. While viral diagnostic has mostly shifted to targeted nucleic acid testing (Polymerase Chain Reaction (PCR), Nucleic Acid Sequence-based Amplification (NASBA)) for years, bacterial diagnostic remains mainly based on culture, and more rarely on the use of molecular tests, such as PCR and antigen detection. The positive predictive value of bacterial culture is very high, and, additionally, bacterial isolation allows for antibiotic resistance phenotyping. Nevertheless, bacterial culture is not a perfect diagnostic tool. For example, a lot of blood cultures remain negative in immunocompromised patients suspected of infection; even in the cases where bacterial or fungal sepsis is likely, negative results are often recorded for blood samples. Quantitative bacterial loads determination seems clinically valuable but is not routinely performed, as it is time and resource consuming. Finally although it has decreased with advanced blood culture techniques, time to results is inconstant, from two days to more than more than one week, and requires additional time for antibiotic resistance determination. New tools, like Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF), speed up identification of bacterial species after the cultivation step but do not profoundly modify the intrinsic limits of cultivation assays.

The advantages of targeted molecular diagnostic are numerous: speed, low cost, automation, sensitivity and specificity, but indeed targeted molecular diagnostic is only able to identify predefined targets. Moreover, for highly variable pathogens like RNA viruses or DNA viruses that comprise multiple genotypes, conserved loci targeted by these tests often do not discriminate between types. Very recently new molecular tests for direct biological sample testing have been released: they use specific multiplex amplifications with a narrow or very broad range to detect a list of agents, defined by syndromes or taxonomy (e.g. bacteria vs. viruses). These tests enlarge the landscape of molecular testing and might represent a major advance, even if sequence-dependent amplification difficultly covers the complexity of etiologies of infections in some indications, like infections of immunocompromised people, and require a frequent adaptation of the panel interrogated.

Non-targeted molecular identification of microbes is now possible based on Next Generation Sequencing techniques, which allows deep sequencing of biological samples, data mining and sorting out of sequences of pathogens without a priori. In this strategy also known as whole genome NGS (WG-NGS), amplification of nucleic acids is random. Advantages of WG-NGS is also its ability to provide more detailed taxonomic information than diagnostic PCRs, as viral subtypes or serotypes. It may also lead to the discovery of new pathogens. The presentation will show power and pitfalls of WG-NGS, and will illustrate its use in use in routine diagnosis.

In fact, WG-NGS was first seen as a research tool due to its initial cost, complexity, and lack of standardization. More recently, we and others have shown that improvements of the sample preparation and bioIT pipelines allow actionable diagnostics in a short and constant time. These studies have shown the medical benefit of WG-NGS as a 'last intention' test in patients who tested negative by standard diagnostic, leading to adapted therapy. In all cases, positive results were not due to the discovery of new pathogens, but to the identification of known pathogens not considered by the physicians as possible etiology or for which corresponding diagnostic tests were not available in clinical laboratories. So, one of the first diagnostic applications of WG-NGS could be testing samples that remain negative with routine diagnostics either for technical or practical reasons, or because of they were not suspected by the clinicians, and so being used as an additional diagnostic tool with an added value.

We also recently undertook a study to evaluate the usefulness of WG-NGS as a 'first-intention' test by comparison with standard pipelines of laboratory in such a population of immunocompromised patients with very encouraging results and will show corresponding results.

Powerful and automated sample preparation pipelines, sequencers in central laboratories and validated pipelines for read sorting and taxonomic assignation are expanding rapidly, in parallel with decreasing costs. The progressive awareness of physicians of the benefit for patients will certainly help WG-NGS to become standard in the practice of conducting microbiological diagnosis in some indications.

Keynote abstracts

Abstract number: 87
(Session 1B)

The Miniaturization of Molecular Technologies for Infectious Disease Testing
Dr. R.L Hodinka
University of South Carolina School of Medicine Greenville, Greenville, South Carolina, United States of America

The global burden of infectious diseases is enormous, resulting in considerable sickness and death, particularly in the very young, the elderly, and individuals with underlying comorbid conditions. Detection and monitoring of microbial pathogens is of prime importance, but the availability of rapid and accurate tests for all laboratories regardless of size, resources, or capacity has been challenging. Molecular testing for infectious disease pathogens is rapidly changing and is being downsized and simplified beyond our wildest imagination. Advances in microelectronics, microfluidics, and microfabrication have paved the way for smaller and simpler molecular platforms and have allowed for more efficient and simultaneous interrogation of a single specimen for multiple pathogens and markers of antimicrobial drug resistance. The ultimate goal of this new molecular revolution is to have self-contained, fully integrated sample-to-report devices that accept raw, untreated specimens; perform all of the molecular steps; and provide interpreted test results within minutes. The hope is that assay miniaturization will lead to greater portability, lower costs, use less sample and reagents, provide for testing at the point of care, result in faster turnaround times, expand test availability, and improve the care and management of patients. With this talk, I will explore the latest advances in molecular technology for rapid and accurate infectious disease testing, describe some of the issues surrounding these methods, and discuss the overall impact of these newer tests on patient care and health outcomes.

Keynote abstracts

Abstract number: 81
(Session 2)

Experiences from the Network of Genomic Medicine (NGM)
Prof. dr. R Büttner, J Wolf
Cologne University Hospital and Center for Integrated Oncology (CIO), Cologne, Germany

Traditionally, tumors are classified by histopathological criteria, i.e., based on their specific morphological appearances. Consequently, current therapeutic decisions in oncology are strongly influenced by histology rather than underlying molecular or genomic aberrations. The increase of information on molecular changes however, enabled by the Human Genome Project and the International Cancer Genome Consortium as well as the manifold advances in molecular biology and high-throughput sequencing techniques, inaugurated the integration of genomic information into disease classification. We have therefore introduced multiplex deep sequencing of informative gene sets into routine histopathological diagnostics and molecular pathology. This comprehensive approach integrating morphological and molecular information is currently changing cancer diagnostics in five categories: (1) Somatic genomic or epigenomic alterations acquired during cancerogenesis may be used for disease classification as we show this approach adding important information to conventional morphological classifications. (2) A significant portion of solid tumors depend on oncogenic driver lesions, which provide molecular targets for prediction of effective and selective therapies. (3) Genomic alterations in signal transduction cascades and gene expression pattern may be used as prognostic parameters predicting the need and extent of adjuvant therapy. (4) In the case of multiple syn- or metachronous tumors, genomic profiling assists allocation of metastases from tumors with unknown primary (CUP) and correct staging as multiple small primary tumors and systemic metastases are reliable being discriminated. (5) Finally, mutational profiling of tumor circulating tumor DNA may facilitate monitoring the response of tumors to therapy and development of secondary resistance.

Taken together, comprehensive molecular tumor pathology and oncology paves the way for a new rational and the basis of personalized genomic medicine. This requires state-of-the art tumor diagnostics and therapies in an interdisciplinary approach. Therefore, we will review current technology and applications of NGS for molecular and predictive cancer diagnostics.

References

Changing histopathological diagnostics by genome-based tumor classification. <http://www.ncbi.nlm.nih.gov/pubmed/24879454> Kloth M, Buettner R. Genes (Basel). 2014 May 28;5(2):444-59.

Lessons learned from lung cancer genomics: the emerging concept of individualized diagnostics and treatment. <http://www.ncbi.nlm.nih.gov/pubmed/23589544> Buettner R, Wolf J, Thomas RK. J Clin Oncol. 2013 May 20;31(15):1858-65. Review.

A genomics based classification of lung tumors. Clinical Lung Cancer Genome Project (CLCGP); Network Genomic Medicine (NGM). Seidel D, et al, Wolf J, Buettner R, Thomas RK. Sci Transl Med. 2013 Oct 30;5(209):209ra153.

Comprehensive genomic profiling of small cell lung cancer. George J et al, and Thomas RK. Nature, in revision.

Keynote abstracts

Abstract number: 80
(Session 2)

RNA-seq of tumor-educated platelets enables blood-based pan-cancer, multiclass and molecular pathway cancer diagnostics

Prof. Dr. T. Wurdinger
VU Medical Center / Cancer Center Amsterdam, Amsterdam, Nederland

Blood-based 'liquid biopsies' provide a means for minimally invasive molecular diagnostics, overcoming limitations of tissue acquisition. Early detection of cancer, clinical cancer diagnostics, and companion diagnostics are regarded as important applications of liquid biopsies. Tumor-educated blood platelets (TEPs) are implicated as central players in the systemic and local responses to tumor growth, thereby altering their RNA profile. We determined the diagnostic potential of TEPs by mRNA sequencing of platelet samples. We distinguished patients with localized and metastasized tumors from healthy individuals with 96% accuracy. Across six different tumor types the location of the primary tumor was correctly identified with high accuracy. Also, MET or HER2-positive, and mutant KRAS, EGFR, or PIK3CA tumors were accurately distinguished using surrogate TEP mRNA profiles. These results indicate that blood platelets provide a valuable platform for pan-cancer, multiclass cancer and companion diagnostics, possibly enabling clinical advances in blood-based 'liquid biopsies'. The unprecedented ability of TEPs to pinpoint the location of the primary tumor advances the use of liquid biopsies for cancer diagnostics. Blood platelets are a potential all-in-one platform for blood-based cancer diagnostics, using the equivalent of one drop of blood.

Keynote abstracts

Abstract number: 89
(Session 3)

Whole genome sequencing as a tool for micro-epidemiological investigations, what have we learnt?
Dr. M. Holden
University of St Andrews, St. Andrews, Fife, United Kingdom

Since the very first microbial genomes were completed two decades ago, whole genome sequencing (WGS) has been used to capture and compare the genetic diversity of bacterial isolates. As sequencing technology has advanced, WGS has extended its potential utility from a tool of scientific research to one of clinical diagnostics. The capacity of WGS to distinguish pathogenic isolates differing by as little as a single mutation, and thus provide high-resolution genotyping, have ensured that it has become a powerful tool for epidemiological investigations. The decrease in costs and increase in speed and capacity of sequencing, coupled with progress in bioinformatics tools for processing and visualizing, mean that WGS is being more widely used in the clinical setting. Using phylogenetic methods to investigate the genetic relationships of isolates it is possible to distinguish distantly related isolates from those with a more recent ancestor. In an outbreak investigation this is particularly powerful when coupled with epidemiological data, as it can be used to rule-in and rule-out isolates, and also infer routes of transmission. In this talk I will illustrate how deep sequencing has been used to investigate the population structure and transmission dynamics of healthcare-associated pathogens, and I will also discuss some of the limitations and considerations of the use of this technology for micro-epidemiological investigations.

Keynote abstracts

Abstract number: 94
(Session 3)

Ebola virus outbreak: working in a field diagnostic laboratory in Liberia, 2014-2015
Dr. E. de Wit,
Laboratory of Virology, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT, USA

The ongoing Ebola outbreak in West Africa has already resulted in more than 28,000 suspected, probable and confirmed cases. Simultaneously, malaria remains a large public health burden in the region most affected by the outbreak. Providing rapid, reliable diagnostics is essential to outbreak management. At the request of WHO, an joint CDC/NIH outbreak laboratory was established in Monrovia, Liberia in late August of 2014, to provide laboratory diagnostics for Ebola virus viremia and *Plasmodium spp*. parasitemia. In the 9 months until Liberia was declared Ebola free, approximately 6000 patient samples were processed in this laboratory.
In this talk, Emmie de Wit will provide an overview of the combined efforts of the CDC/NIH laboratory, highlighting the diagnostic procedures for safe, rapid and reliable sample processing, the epidemiological data collected as well as personal experiences of working four months in a field laboratory during an Ebola outbreak

Keynote abstracts

Abstract number: 95
(Session 4)

Title : Molecular markers for detection of colorectal cancer
Prof. dr. M. van Engeland
Dept. of Pathology, GROW-School for Oncology and Developmental Biology, Maastricht University
Maastricht, The Netherlands

Colorectal cancer has predominantly been considered a genetic disease, characterized by sequential accumulation of genetic alterations. Growing evidence indicates that epigenetic alterations add an additional layer of complexity to the pathogenesis of colorectal cancer, and characterize a subgroup of colorectal cancers with a distinct etiology and prognosis. Here I discuss recent data on epigenetic regulation of gene expression in CRC and describe how the understanding of these processes will alter the management of colorectal cancer. I also discuss the challenges that we face in bringing epigenetic markers to the clinic.

Keynote abstracts

Abstract number: 91
(Session 4)

The daunting challenge of molecular HPV testing and biomarker implementation in Cervical Cancer Screening

Dr. J Bonde
Hvidovre Hospital, Denmark

New Molecular Horizons in Cervical Mass-Screening

Cervical screening is rapidly changing face in these years. For more than decade evidence from clinical randomized control trials, split sample trials, implementation trials and implementation pilots have proven that molecular screening for human papillomavirus rather than screening with cytology for cellular abnormalities yields the best and longest protection in women against cervical cancer. That evidence is now being transformed into changing national guidelines. Countries like Holland, Sweden, Norway, Scotland, England Denmark and others are currently underway with replacing cytology fully or partially with primary molecular HPV screening, and most recently, 4th September 2015, the European Guidelines for cervical screening also called for primary HPV screening rather than cytology.

However, are all molecular HPV assays “created equal”? Will knowledge of a given woman´s HPV genotype have any value in risk stratification for follow up? And how do we deal with the many screening false positive HPV tests? How will population HPV vaccination change the requirements of screening, and how can primary HPV screening and self-sampling be combined to offer a 21th century screening solution. Finally, will molecular biomarkers like methylation come into play in order to optimize molecular HPV cervical screening, resulting in a more personalized screening?

To answer these questions, the lecture will report from the Danish HORIZON trial, the EU-VIPER trial, a number of other studies, as well as from the laboratory front lines in the battle against cervical screening.

Keynote abstracts

Abstract number: 88
(Session 5A)

Assuring quality patient testing: Standardization challenges in new and rapidly evolving molecular diagnostics

Dr. R.M. Madej
QCMD, Glasgow, Scotland, United Kingdom

With the increased demand for molecular testing, and the development of user-friendly assay systems, molecular diagnostic tests are becoming more common in medical laboratories. Tests that were performed by scientists who were specifically oriented to - and may have developed the tests for molecular diagnostics- are now accessible to a broader set of professionals. On the other end of the spectrum, increasingly complex technologies and testing strategies such as NGS, predictive algorithms, metabolomics, and pharmacogenomics are rapidly being implemented to provide needed information for directing patient care. Laboratory professionals, clinicians, industry professionals, and regulators are trying to balance the development and maintenance of the necessary, rigorous quality standards for patients without impeding the important healthcare opportunities that this field provides. Meanwhile, the medical laboratory must continue the daily work of delivering the highest quality laboratory test results in the midst of a quickly evolving environment. This session will discuss some of the challenges facing medical laboratories, some resources for best practices and materials, and opportunities for shaping the discussion of continued medical laboratory quality.

Keynote abstracts

Abstract number: 82
(Session 5B)

NGS data analysis, quality aspects for diagnostics
Dr. M Noguera-Julian
IrsiCaixa - AIDS research Institute, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

If untreated, Human Immunodeficiency Virus (HIV) infection leads to progressive immune deterioration and Acute Immunodeficiency syndrome (AIDS) and death. Highly active antiretroviral therapy (HAART) can turn HIV infection into a manageable chronic disease. Standard, Sanger-based, HIV genotyping techniques are recommended to determine HIV resistance in order to guide HAART design. However, these techniques can't detect low frequency drug resistant viral variants (LDRV) and when more sensitive next generation sequencing (NGS) based genotyping is used, quality assessment and analysis tools are required to reliably detect these LDRV from raw sequence data, to assess its clinical value and to build a clinical laboratory diagnostic tool

Keynote abstracts

Abstract number: 92
(Session 6)

'What clinically useful information can whole genome sequencing of rare or complex disease suffering or of healthy individuals provide?'

Dr. E Worthey
Hudson Alpha Institute for Biotechnology, Huntsville, AL, United States of America

Over the last decade, next-generation sequencing (NGS) has transformed genomic research through substantial advances in technology and reduction in the cost of sequencing and analysis of genomic data. Genome wide sequencing (GWS) is now being used as a standard molecular diagnostic test under particular circumstances in some clinical settings. Clearly, it is often the first best test to identify novel or known, pathogenic or likely pathogenic variation associated with rare monogenic disease. In addition it can identify much (though not all) variation associated with more common and polygenic disease and is increasingly being used to provide clinically actionable data for appropriate drug selection in a variety of clinical settings including neurology, oncology, and immunology. More recently, this method has been applied for screening for disease predisposition and risk in apparently healthy individuals. This talk will examine the strengths and challenges of this approach, exploring the types of information that can be identified through its application both in patients with rare or common disease, and alternatively in ostensibly healthy individuals.

Keynote abstracts

Abstract number: 86
(Session 7)

DNA diagnostics for guiding drug therapy: latest developments at the European level
Prof. dr. R van Schaik
Erasmus MC, Rotterdam, Nederland

In the last three years, there has been an increase in the clinical use of pharmacogenetic testing, addressing inherited polymorphisms in drug metabolizing enzymes and transporters affecting enzymatic activities in liver and intestine. These DNA variants affect drug exposure, making them suitable markers to adjust drug therapy up front. Although the promise of this approach was long advocated, there is a growing awareness that this type of diagnostics may indeed greatly improve personalized care. In this presentation, the currently used pharmacogenetic test and the currently ongoing discussions on the research, ethical and political field about their use will be addressed. In addition, the organization of pharmacogenetic implementation at the European level (www.eu-pic.net <http://www.eu-pic.net>, www.u=pgx.eu <http://www.u=pgx.eu>) will be highlighted.

Keynote abstracts

Abstract number: 83
(Session 7)

Pharmacogenomic and epigenomic biomarkers for predicting interindividual differences in drug metabolism and response

Prof. dr. M Ingelman-Sundberg
Karolinska Institutet, Stockholm, Sweden

There are pronounced interindividual variations in drug metabolism, drug response and incidence of adverse drug reactions. In addition to genetic variation, epigenetic dependent regulation of these genes is important and future direction in this novel research field is outlined with respect to our understanding of interindividual differences in drug action (Ivanov et al., 2014). Regarding the genetic variation it is clear that in addition to common previously characterized variations of importance for drug response (ingelman-Sundberg, 2015) which are frequently utilized for current therapy (Ehmann et al., 2015), there are also a huge number of rare gene variants of importance for the individual response worth attention (Fujikura et al., 2015). A novel class of drugs, so called epidrugs, are known to can intervene in the epigenetic control of gene expression for disease treatment, and many so called epidrugs are now in clinical development. In addition, disease diagnosis prognosis and drug treatment success can be monitored by epigenetic biomarkers. The lecture will give an update in the field of current and future genomic and epigenomic biomarkers, and epigenetic mechanisms of importance for prediction of drug metabolism, drug action and ADRs focusing on the most clinically relevant examples.

Ehmann F, Caneva L, Prasad K, Paulmichl M, Maliepaard M, Llerena A, Ingelman-Sundberg M, Papaluca-Amati M. Pharmacogenomic information in drug labels: European Medicines Agency perspective. Pharmacogenomics J. 2015;15:201-10.

Fujikura K, Ingelman-Sundberg M, Lauschke VM. Genetic variation in the human cytochrome P450 supergene family. Pharmacogenet Genomics. 2015 Sep 3. Epub ahead of print]

Ingelman-Sundberg M. Personalized medicine into the next generation. J Intern Med. 2015;277:152-4.

Ivanov M, Barragan I, Ingelman-Sundberg M. Epigenetic mechanisms of importance for drug treatment. Trends Pharmacol Sci. 2014;35:384-96

Keynote abstracts

Abstract number: 79
(Session 8)

Labs-on-a-Chip for medical applications
Prof. dr. Ir. A van den Berg
University of Twente, Twente, Nederland

The recent rapid developments in microfluidics technologies has enabled the realization of miniaturized laboratories. These Labs-on-a-Chip will play an important role in future medicine, both in point-of-care devices for drug or biomarker monitoring, as well as in early diagnostic devices. We developed a pre-filled ready-to-use capillary electrophoresis platform for measuring ions in blood. It is used to monitor lithium in finger-prick blood of manic-depressive patients, but can also be used for measuring calcium in blood for prevention of milk fever, or for measuring creatinine in blood or sodium in urine for early detection of ESRD. Another device was developed for analyzing male fertility by determining sperm concentration and motility in semen. It appears that the same device can be easily adapted to detect the presence of cells in milk, a good indicator for the presence of mastitis.For early detection of colon cancer, nanowire sensors for detection of hypermethylated DNA will be presented, showing label-free DNA detection. The small size of these nanowire sensors enables the integration of a complete lab in a pill, that may be used as a screening tool for early diagnostics of intestinal cancer. Another example of nanostructured sensors are nanopiramids that are used for SERS detection, allowing direct, labelfree detection and identification of biomarkers. Finally, some perspective for microfluidic devices for permeation studies of tissue and organs on chip will be discussed.

Keynote abstracts

Abstract number: 96
(Session 8)

Differential amplicons (ΔAmp) - a new method for quality assessment of RNA in Molecular Diagnostics
Mr. J. Björkman[1], David Svec[1,2], Emelie Lott[1], Mikael Kubista[1,2], Robert Sjöback[1]
[1]TATAA Biocenter AB
[2]Laboratory of Gene Expression, Institute of Biotechnology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Since the publication of the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines in 2009 users' awareness and clients' requests for quality control (QC) in qPCR has dramatically increased. To comply with MIQE professional providers of qPCR services and contract research organizations emphasize the different aspects of quality control in their offerings. TATAA Biocenter co-authored the MIQE- guidelines, was partner in the SPIDIA consortium (www.spidia.org), member of the workgroup drafting the forthcoming ISO guidelines for the pre-analytical steps in molecular diagnostics, and is certified according to ISO 17025. TATAA early identified the need for and importance of stringent quality control in qPCR and has focused on developing methods and tools to asses sample, assay and performance quality parameters. In this work we have identified an Endogenous RNase Resistant (ERR) marker that can be used to assess RNA quality in samples exposed to temperatures and conditions at which nucleases are active or freeze-thawing. We also developed protocols to assess physical and chemical degradation, such as damage caused during tissue fixation. The ΔAmp method will be presented demonstrating its advantages in terms of sensitivity, performance and ease of use compared to traditional methods such as capillary electrophoresis.

Related publications:

J. Björkman, D. Svec, E. Lott, M. Kubista, R. Sjöback, Differential amplicons (ΔAmp)—a new molecular method to assess RNA integrity, Biomolecular Detection and Quantification (2015), http://dx.doi.org/10.1016/j.bdq.2015.09.002 (article in press)

Hui Zhang, Vlasta Korenkova, Robert Sjöback, David Svec, Jens Björkman, Mogens Kruhøffer, Paolo Verderio, Sara Pizzamiglio, Chiara Maura Ciniselli, Ralf Wyrich, Uwe Oelmueller, Mikael Kubista, Torbjørn Lindahl, Anders Lönneborg, Edith Rian, Biomarkers for Monitoring Pre-Analytical Quality Variation of mRNA in Blood Samples PlosOne, 9(11), e111644 (November 2014)

Kashofer K, Viertler C, Pichler M, Zatloukal K (2013) Quality Control of RNA Preservation and Extraction from Paraffin-Embedded Tissue: Implications for RT-PCR and Microarray Analysis. PLoS ONE 8(7): e70714. doi: 10.1371/journal.pone.0070714

Stephen A Bustin et al. The need for transparency and good practices in the qPCR literature. Nature Methods 11:1063-1067 (November 2013)

F. Malentacchi, M. Pazzagli, L. Simi, C. Orlando, R. Wyrich, C.C. Hartmann, P. Verderio, S. Pizzamiglio, C.M. Ciniselli, A. Tichopad, M. Kubista, S. Gelmini. SPIDIA-DNA: An External Quality Assessment for the pre-analytical phase of blood samples used for DNA-based analyses. Clinica Chimica Acta 424, 274–286 (2013)

M. Pazzagli, F. Malentacchi, L. Simi, C. Orlando, R. Wyrich, K. Günther, C.C. Hartmann,P. Verderio, S. Pizzamiglio, C.M. Ciniselli, A. Tichopad, M. Kubista, S. Gelmini. SPIDIA-RNA: First external quality assessment for the pre-analytical phase of blood samples used for RNA based analyses. Methods 59, 20-31 (2013)

Mikael Kubista, Jens Björkman, David Svec, and Robert Sjöback, RNA quality matters European_Pharmaceutical_Reviews Vol 17, Issue 6, 2012

Stephen A. Bustin, Vladimir Benes, Jeremy A. Garson, Jan Hellemans, Jim Huggett, Mikael Kubista, Reinhold Mueller, Tania Nolan, Michael W. Pfaffl, Gregory L. Shipley, Jo Vandesompele,5 and Carl T. Wittwer. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55,4 (2009).

ORAL ABSTRACTS BY ORDER OF ABSTRACT NUMBER

Oral abstracts

Abstract number : 4

PACBIO SEQUENCING OF THE GENETIC DIVERSITY OF THE HEPATITIS C VIRUS ENVELOPE REGION: FROM EARLY ACUTE TO CHRONIC INFECTION

R Molenkamp[1], K.Y. Ho[1], J Raghwani[2], S.M. Koekkoek[1], M.D. de Jong[1], O.G. Pybus[2], C.J. Schinkel[1]
[1]AMC, AMSTERDAM, Netherlands
[2]University of Oxford, OXFORD, United Kingdom

Deep sequencing has revolutionized the study of genetically variable RNA virus populations, but for phylogenetic and evolutionary analyses longer sequence length and low error rates are desired. The Pacific Biosciences Single Molecule, Real Time (SMRT) sequencing approach provides long reads and circular consensus sequences (CCS). We investigated using Pacbio sequencing the evolution of the hepatitis C virus (HCV) envelope region (E1E2, 1680 bp) in five subjects with incident infection who progressed to chronicity.

The five subjects were infected with closely related HCV genotype 4d variants and coinfected with HIV-1. Four subjects were men who have sex with men (MSM) and the 5th subject was the female partner of one of the MSM. Fifty samples, collected between 2001-2013, were SMRT sequenced. The sequencing error at 7 CCS full passes was 0.37% with insertions as the main type of error (0.24%), followed by deletions (0.11%). Mismatches were surprisingly low (0.02%). The median coverage at 7 full passes was 612 CCS reads/sample (range 149-935). Prior to phylogenetic analysis, insertions with respect to a sample-specific reference sequence were removed. Neighbor Joining phylogenies revealed a close phylogenetic relationship between reads from the four MSM at early time points, and evidence for transmission from one MSM to the female subject. Intra-host phylogenies of reads sampled early during infection suggest that a single founder virus established infection in all five subjects. This finding was supported by the low genetic diversity observed at these early time points. The increase in diversity coincided with progression to chronicity and the emergence of multiple co-existing lineages. Changes in the genetic diversity during chronic infection corresponded with a non-ladder like phylogeny.

SMRT sequencing is able to combine great coverage with long reads and can provide rich insights into HCV dynamics from transmission bottlenecks to long-term chronic infection.

Oral abstracts

Abstract number : 9

Transmission of high risk K. pneumoniae clones in health care networks largely challenges the current infection prevention and control system

J.W.A. Rossen, M Lokate, RH Deurenberg, M Tepper, JP Arends, GC Raangs, J Lo-Ten-Foe, H Grundmann, K Zhou, AW Friedrich
UMCG, GRONINGEN, Nederland

Controlling dissemination of multidrug-resistant pathogens remains one of the major public health challenges. Here we describe an inter-institutional transmission of an ESBL-producing ST15 *Klebsiella pneumoniae* between patients caused by patient referral. A suspected epidemiological link between clinical isolates was supported by patient contact tracing and phylogenetic analysis of isolates obtained from May to November 2012 using next generation sequencing (NGS). By May 2013, a patient treated in two institutions in two cities was involved in the expanding cluster. A clone-specific multiplex PCR was developed for patient screening by which another patient was identified in September 2013. Environmental surface contamination and lack of consistent patient screening were identified as risk factors. Our study highlights the challenge of controlling the transmission of *K. pneumoniae* high risk clones (HiRiCs), suggesting the necessity for active surveillance and inter-institutional collaboration for outbreak management. In addition, the use of NGS for typing and for developing an outbreak-specific multiplex PCR facilitated rapid patient screening procedures and was important for optimizing outbreak management.

Oral abstracts

Abstract number : 13

CERVICAL CANCER SCREENING IN THE NETHERLANDS: DETERMINATION OF HPV PREVALENCE USING THREE DIFFERENT SYSTEMS.

C.J.J. Huijsmans
Jeroen Bosch Hospital, 'S-HERTOGENBOSCH, Netherlands

C.J.J. Huijsmans[1], W.R. Geurts-Giele[1,2], C. Leeijen[1], E. Hazenberg[1], D. Mulder[3], J.C. van der Linden[1], A.J.C. van den Brule[1]
Pathologie-DNA, [1]location Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands; [2]Erasmus MC Cancer Institute, Department of Pathology, Rotterdam, The Netherlands; Pathologie-DNA, [3]location Rijnstate Hospital, Arnhem, The Netherlands.

Objectives. Primary high risk (hr)HPV screening will be introduced in The Netherlands during the 2nd quarter of 2016. Our aim was to determine the hrHPV prevalence in a representative patient cohort. In addition, the workflow will also be studied.
Methods. A total of 11,802 residual PreServCyt cervical samples from the Dutch population based cytology screening program were rendered anonymous, randomized and tested for hrHPV using 3 completely automated HPV detection systems: Qiagen Hybrid Capture 2 (HC2), Roche Cobas4800 and Hologic Aptima.
Results. The selected samples were representative for the population based screening program with respect to age distribution and cytology classification. Employing the 3 systems, the mean hrHPV prevalence was 7.9±0.3% and therefore, independently of the assay used, higher than the previously reported 4-5% using the GP5+/6+PCR-EIA (POBASCAM) and HC2 (VUSA-Screen) HPV tests. As expected, a clear age dependency was found, with an hrHPV prevalence ranging from 18.6±1.1% in women 29-33 years of age to 3.9±0.3% in women 59-63 years of age. Also for severity of cytology a correlation with hrHPV prevalence was observed, ranging from 5.4±0.3% in normal cytology to 92.2±3.1% in severe dysplasia.
Conclusions. In contrast to the report of the Dutch Health Council, a higher hrHPV prevalence of 7.9±0.3% was found in this population based screening cohort using the complete HPV detection systems from Qiagen (HC2), Roche (Cobas4800), and Hologic (Aptima), which has consequences for the cost-effectiveness of the Dutch screening program. Additionally, the workflow and workload of the 3 hrHPV testing solutions were found to differ significantly.

Oral abstracts

Abstract number : 15

Development of 2 multiplex real-time PCR assays for the detection of the Celiac disease related Humaan Leukocyte Antigens DQ2.5, DQ2.2 en DQ8

M Oostra, IJM Gotink, JJJ Hulstein
Gelre hospitals, APELDOORN, Netherlands

Celiac disease is an autoimmune disease in which ingestion of gluten, present in wheat, barley and rye, leads to damage of the small intestine resulting in chronic diarrhea. Approximately 90 percent of Celiac disease patients carry a MHC class II human leukocyte antigen (HLA) variant called HLA-DQ2.5 and the remaining 10 percent carries either an HLA-DQ8 or HLA-DQ2.2 variant. These variants are also found in approximately 40% of the general population. However screening for these variants is of great diagnostic value since their absence has a very good negative predictive value.
We have developed 2 multiplex real-time PCR assays for the detection of the HLA-DQ2.5, -DQ2.2 and -DQ8 alleles. One multiplex detects the alleles DQA1*05, DQA1*02 and DQB1*02 to demonstratie the presence of HLA-DQ2.5 or -DQ2.2. The second multiplex detects the alleles DQA1*03 and DQB1*0302 to demonstrate the presence of HLA-DQ8. Both assays also detect a household gene as a control.
Retrospectively we tested 45 samples in the real-time PCR assay of which the HLA-DQ alleles were determined by Luminex SSO in combination with PCR-SSP (by Sanquin). No discrepancies were found in the detection of the presence or absence of the HLA-DQ2.5, -DQ2.2 or -DQ8 variants. Next, 30 samples were compared prospectively and also here no discrepancies were found.
Therefore we conclude that the developed multiplex real-time PCR assay has a good negative predictive value for the presence of the Celiac disease related HLA-DQ variants. Furthermore the assay is easy to perform and analyse and relatively cheap. This assay can thus very well be used als primairy screening in the diagnosis of Celiac disease.

Oral abstracts

Abstract number : 17

Micelle PCR reduces artifact formation during 16S rRNA microbiota profiling

R Jansen[1], S. A. Boers[2], J. P. Hays[2]
[1]Streeklab Haarlem, HAARLEM, Netherlands
[2]Erasmus medical centre, ROTTERDAM, Nederland

16S rRNA gene profiling has revolutionized the field of microbial ecology. Many researchers in various fields have embraced this technology to investigate the bacterial composition of samples derived from many different ecosystems. However, it is important to appreciate the current limitations and drawbacks of 16S rRNA gene profiling. Although sample handling, DNA extraction methods and the choice of universal 16S rRNA gene PCR primers, are well known factors that could seriously affect the final results of microbiota profiling studies, inevitable amplification artifacts, such as chimera formation and PCR competition, are seldom appreciated. Here we report on a novel micelle based amplification strategy using the V3-V5 region of the 16SrRNA gene, which overcomes these limitations via the clonal amplification of target DNA molecules. Micelle PCR is a single-molecule clonal amplification method in which template DNA molecules are separated into a large number of physically distinct reaction compartments using a water in oil emulsion. Our results show that micelle PCR drastically reduces chimera formation by a factor of 38 (1.5% vs. 57%) compared with traditional PCR, resulting in strongly improved microbial diversity estimates as demonstrated by a synthetic mix of 20 bacterial species. In addition, compartmentalization during micelle PCR prevents the generation of PCR competition artifacts which may be generated due to the unequal amplification rate of different 16S rRNA template molecules, generating more robust and accurate 16S rRNA microbiota profiles.
Currently, we are employing the micelle PCR to analyze infected sites of human patients and compare the data with routine diagnostic culturing techniques.

Oral abstracts

Abstract number : 29

BRAF and KRAS are not mutually exclusive: next generation sequencing reveals two cases of concomitant KRAS and BRAF mutation in metastatic colorectal cancer

J Breyne, A Vandamme, E De Laere, F Dedeurwaerdere
AZ Delta Roeselare, ROESELARE, Belgium

Introduction
Mutations in KRAS or NRAS exon 2, 3 or 4 are validated biomarkers of resistance to anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer (mCRC). 8-15% of mCRC tumors harbor BRAF mutations, of which the impact on the efficacy of anti-EGFR therapy remains controversial. Mutations in KRAS, NRAS and BRAF are considered mutually exclusive. Routine laboratory testing is based on that theory: KRAS mutation analysis is performed, followed by NRAS mutation analysis on wild-type KRAS tumors and finally BRAF mutation analysis on wild-type RAS tumors, limiting the chance of discovering co-occuring mutations. In the present study, next generation sequencing (NGS) analysis was performed on mCRC samples, testing the three parameters in the same analysis. We here report our first special cases.
Methods
NGS analysis is performed on a GS-Junior+ sequencer (Roche), using in-house PCR for target amplification (KRAS exon 2, 3 and 4; NRAS exon 2, 3 and 4; and BRAF exon 11 and 15) and 454 MID primers (Multiplicom) for sample identification. Sequencing analysis was performed following manufacturer's instructions. Data was analyzed using the Amplicon Variant Analyzer software (Roche).
Results
From February until June 2015, 66 patients with mCRC were tested for the presence of KRAS, NRAS or BRAF mutations using NGS. Forty tumors harbored a mutation in either BRAF, KRAS or NRAS. Two patients showed concomitant KRAS (exon 2) and BRAF (exon 11) mutation. Due to the infrequent observation of this phenomena, it is not clear whether or not these tumors have a different biology than tumors with a single mutation, or which of the two is the dominant oncogene.
Discussion
Using NGS, we predict more frequent observation of co-occuring mutations in mCRC. These findings offer a new dimension in tumor genetics, where mutations involving multiple cancer genes may reveal new strategies for therapeutic intervention.

Oral abstracts

Abstract number : 36

EVALUATION OF THE IDYLLA BRAF MUTATION ASSAY PERFORMANCE USING THE NOVEL FULLY-AUTOMATED IDYLLA SYSTEM ON FORMALIN-FIXED PARAFIN EMBEDDED MELANOMA SAMPLES

MI Micalessi, J Frans, V Noten, I Van Hee, C Goris, W Wynendaele, C Bourgain
Imelda Hospital, BONHEIDEN, Belgium

Mutations in the BRAF gene are found in diverse cancers such as melanoma, papillary thyroid and colorectal cancers. Targeted therapy with BRAF and MEK inhibitors has the potential for rapid tumour regression in patients whose melanoma harbours a BRAF mutation. In our daily routine, the assessment of tumour mutation status involves shipment of the paraffin block from our local pathology laboratory to a dedicated molecular pathology center. The Idylla™ system (Biocartis), which is a novel fully-automated molecular diagnostics platform, offers the possibility to perform the BRAF mutation analysis in our laboratory in a short time (90 min) and with minimal hands-on time (<2 min/FFPE section).
Therefore, this study aims to validate the analytical performance of the Idylla™ BRAF Mutation test (Biocartis) on FFPE specimens using the Idylla™ System.
The analytical performance of the Idylla™ assay was determined through the evaluation of the following parameters: limit of detection (LOD with 95% hit rate), specificity, accuracy and precision. The Idylla™ assay detected the BRAF mutant alleles which were present in the reference standards at a frequency of 1% on different testing days and this demonstrates the high inter-run reproducibility and sensitivity of the assay.
Accuracy and specificity of the Idylla™ assay was assessed through the analysis of 6 external quality control samples and 19 FFPE samples from melanoma tumours. The obtained results correlated highly with the reference methods results.
To conclude, our findings demonstrate that the Idylla ™ System is a fast, sensitive and reliable method to determine the BRAF mutation status in FFPE melanoma tumour samples.

Oral abstracts

Abstract number : 37

EVALUATION OF THE IDYLLA BRAF MUTATION ASSAY PERFORMANCE USING THE NOVEL FULLY-AUTOMATED IDYLLA SYSTEM ON FORMALIN-FIXED PARAFIN EMBEDDED COLORECTAL CANCER SAMPLES

MI Micalessi, J Frans, V Noten, I Van Hee, C Goris, PJ Cuyle, C Bourgain
Imelda Hospital, BONHEIDEN, Belgium

Mutations in the BRAF gene are found in diverse cancers such as melanoma, papillary thyroid and colorectal cancers. Targeted therapy with BRAF inhibitors or anti-EGFR monoclonal antibodies has the potential for rapid tumour regression in patients whose colorectal carcinoma does not harbour a BRAF mutation. In our daily routine, the assessment of tumour mutation status involves shipment of the paraffin block from our local pathology laboratory to a dedicated molecular pathology center. The Idylla™ system (Biocartis), which is a novel fully-automated molecular diagnostics platform, offers the possibility to perform the BRAF mutation analysis in our laboratory in a short time (90 min) and with minimal hands-on time (<2 min/FFPE section).
Therefore, this study aims to validate the analytical performance of the Idylla™ BRAF Mutation test (Biocartis) on FFPE specimens using the Idylla™ System.
The analytical performance of the Idylla™ assay was determined through the evaluation of the following parameters: limit of detection (LOD with 95% hit rate), specificity, accuracy and precision. The Idylla™ assay detected the BRAF mutant alleles which were present in the reference standards at a frequency of 1% on different testing days and this demonstrates the high inter-run reproducibility and sensitivity of the assay.
Accuracy and specificity of the Idylla™ assay was assessed through the analysis of 6 external quality control samples and 10 FFPE colorectal cancer specimens. The obtained results correlated excellently with the reference methods results.
To conclude, our findings demonstrate that the Idylla™ System is a fast, sensitive and reliable method to determine the BRAF mutation status in FFPE colorectal tumour samples.

Oral abstracts

Abstract number : 38

Comparison of digital PCR platforms and semi-nested qPCR as a tool to determine the size of the HIV reservoir

K.J. Bosman[1], M Nijhuis[1], P.M. van Ham[1], A.M.J. Wensing[1], K Vervisch[2], L Vandekerckhove[2], W De Spiegelaere[2]
[1]UMC Utrecht, UTRECHT, Netherlands
[2]University Hospital Gent, GENT, Belgium

HIV persists in latently infected cells of patients on antiretroviral therapy (ART). This persistent proviral DNA reservoir is an important predictor of viral rebound upon therapy failure or interruption and forms a major obstacle towards cure. Accurate quantification of the low levels of persisting HIV DNA may aid patient monitoring and cure research. Digital PCR is a promising tool that enables direct absolute quantification with high sensitivity. With recent technological advances, several platforms are available to implement digital PCR in a clinical setting. Here, we compared two digital PCR platforms, the Quantstudio 3D (Life Technologies) and the QX100 (Bio-Rad) with a semi-nested qPCR on serial HIV DNA dilutions and DNA isolated from PBMCs of ART-suppressed patients.
All three methods were able to detect target to the lowest levels of 2.5 HIV DNA copies. The QX100 excelled in having the least bias and highest precision, efficiency and quantitative linearity. Patient sample quantifications by the QX100 and semi-nested qPCR were highly agreeable by Bland-Altman analysis (0.01 ± 0.32 $\log_{10}$). Due to the observation of false-positive signals with current digital PCR platforms however, semi-nested qPCR may still be preferred in a setup of low quantity detection to discriminate between presence or absence of HIV DNA.

Oral abstracts

Abstract number : 40

Hand-held Point-of-Care Test Device for the Rapid Detection of Infectious Diseases

B Mahony, B Stone
McMaster University, HAMILTON, Canada

Background: Most point-of-care test (POCT) devices detect antigens or antibody; however, these assays are insensitive compared with nucleic acid detection methods. Therefore there is an urgent need for nucleic acid amplification based POCT tests for the detection of infectious diseases. We describe here an instrument-free, hand-held, POCT device that can detect viruses and bacteria on a swab providing an answer in 20 minutes.
Methods: Our POCT device is self-contained and does not require an additional analyzer. A patient swab is inserted into the hand-held POCT device which performs the following procedures: elution of the material off the swab, lysis of viral and bacterial pathogens, isothermal amplification of nucleic acid gene targets, and detection of amplified DNA by either a visible color change or by electrochemical methods providing a test result in 20 minutes.
Results: We have developed 35 different assays for use in the device all of which have a lower limit of detection of 1-10 genome equivalents; these include influenza, RSV, Dengue virus, Ebola virus and *C. trachomatis*. To date our POCT device has detected the following pathogens on patient swabs: Influenza A and B, RSV A and B in nasal swabs, *Chlamydia trachomatis* in vaginal swabs and first catch urines, and Group A streptococcus in throat swabs. The hand-held device provided an accurate detection of the infectious agent in under 20 minutes permitting the immediate initiation of appropriate treatment.
Conclusions: We have developed a hand-held POCT device that accepts a patient swab and detects the presence of specific infectious agents (viruses and bacteria) in 20 minutes. The device is completely self-contained, is disposable and does not require an analyzer. This hand-held device can be used by non-health care workers in both clinical and non-clinical settings where there is no laboratory support providing a swab-to-result diagnosis in 20 minutes.

Oral abstracts

Abstract number : 46

Assessing the public health benefit of defective-interfering influenza viruses

M Jonges[1], B Rockx[1], A Meijer[1], MPG Koopmans[2]
[1]National Institute for Public Health and the Environment, BILTHOVEN, Nederland
[2]Department of Viroscience, Erasmus MC, ROTTERDAM, Nederland

Marcel Jonges[1,2], Barry Rockx[1], Adam Meijer[1], Marion Koopmans[1,2]
[1] Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[2] Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands

Defective influenza virus RNA segments, characterized by loss of a central region of the parental RNA segment, compete with full-length viral gene segments at the step of virion assembly and thereby contribute to the production of non-infectious progeny virus. Subsequent virions that carry such an incomplete genome are known as defective-interfering (DI) viruses. Due to the unsuccessful detection of DI genomes *in vivo*, it was commonly believed that DI viruses were laboratory artifacts that typically occurred at a high multiplicity of infection. Previously, we were able to detect and characterize DI viruses in avian influenza virus positive poultry samples using a Next Generation Sequencing (NGS) approach. Here, we further explore the presence of DI viruses in influenza virus positive poultry samples, and characterize the dynamics of defective influenza virus PB2 segments during infection using NGS. In addition, the occurrence of DI viruses in influenza virus positive human respiratory samples was evaluated. A real-time reverse transcriptase PCR was developed targeting defective influenza virus PB2 segments. Conventional Sanger sequencing was applied for both confirmation and characterization of the *in vivo* derived defective PB2 segments.
In conclusion, we describe presence of DI influenza viruses in clinical samples of both poultry and humans. Current molecular epidemiological approaches for transmission tree reconstruction utilize sequence data from influenza virus RNA segments. Our data shows that due to the dynamic nature of defective influenza virus RNA segments during infection, characterized by highly variable deletions derived from parental RNA segments, defective RNA segments can provide additional resolution for the robust characterization of transmission events.

Oral abstracts

Abstract number : 47

Highly multiplexed assay for detection of antimicrobial resistance using PASS MNAzyme qPCR: Mycoplasma genitalium and macrolide resistance

E Mokany[1], LY Tan[1], S Walker[1], M Poljak[2], J Twin[3], S Garland[3], C Bradshaw[4], CK Fairley[4], SN Tabrizi[2]
[1]SpeeDx Pty Ltd, EVELEIGH, Australia
[2]The Royal Children's and The Royal Women's Hospitals, MELBOURNE, Australia
[3]Murdoch Children's Research Institute, MELBOURNE, Australia
[4]Melbourne Sexual Health Centre, MELBOURNE, Australia

Increased macrolide use has been associated with the emergence of antibiotic resistance in *M. genitalium* (Mg) and ineffective cure rates. Resistance has been linked to mutations at two positions, 2058 and 2059 (*E. coli* numbering) in the Mg *23S rRNA* gene. Clinical samples diagnosed with Mg were evaluated with a combined-diagnostic resistance assay that employs novel PASS primers coupled with MNAzyme detection. PASS primers selectively amplify mutant over wild-type and MNAzymes allow for efficient detection and discrimination of multiple mutations simultaneously. Features unique to PASS MNAzyme qPCR allow "stacking"of the 5 SNP assays for a single readout. Multiplexed PASS MNAzyme qPCR was evaluated by comparison to previously screened clinical samples for Mg (*MgPa* gene) and 23S mutations using Sanger sequencing and HRM analysis. Using artificial templates, this assay was able to detect mutation templates ranging from 10-10,240 copies/reaction, with an associated Mg detection limit comparable to existing assays used in routine diagnostics. Preliminary screening of DNA from 24 clinical samples revealed Mg detection range of 3-300,000 copies/reaction. Multiplexed PASS MNAzyme qPCR offers the ability for simultaneous detection of Mg and macrolide resistance mutations. This assay offers considerable advantages in clinical settings with rapid identification of macrolide resistant strains and the ability to implement effective second line agents without delay.

Oral abstracts

Abstract number : 48

EVALUATION OF A RAPID EBOLA VIRUS TRIAGE TEST ON THE IDYLLA™ SYSTEM

L Cnops[1], L HEYNDRICKX[1], B DE SMET[2], M VAN ESBROECK[1], D NAUWELAERS[3], R THIJS[3], G MEERSSEMAN[3], S VAN DEN HERREWEGEN[3], P VAN DEN EEDE[4], J BADEN[5], N VERGAUWE[3], KK ARIËN[1]
[1]Institute of Tropical Medicine, ANTWERP, Belgium
[2]Institute of Tropcial Medicine, ANTWERP, Belgium
[3]Biocartis, MECHELEN, Belgium
[4]Janssen Diagnostics BVBA, BEERSE, Belgium
[5]Janssens Diagnostics LLC, RARITAN, United States of America

The ebola outbreak of 2014 in West-Africa is by far the largest and most geographically widespread Ebola virus disease (EVD) epidemic since the virus was first discovered in 1976. Current EVD diagnosis relies on reverse transcription-PCR (RT-PCR) technology, requiring skilled laboratory personnel and technical infrastructure. In the scope of its function as national reference laboratory for infectious and tropical diseases, the Institute of Tropical Medicine (I) in Antwerp, implemented molecular tests to provide rapid Ebola diagnosis in Belgium. With the unprecedented scale of the current epidemic in West Africa, the development of new diagnostic tests has been accelerated. Rapid, safe and easy-to-use assays for accurate diagnosis of EVD are needed. Biocartis (Mechelen, Belgium) developed a prototype Ebola Virus triage test in association with the I™ and Janssen Diagnostics for its Idyll™ system. This fully automated sample-to-result molecular diagnostic test has a turnaround time of 100 minutes with minimal sample handling. The prototype Ebola Virus triage test simultaneously detects Ebola virus species Zaire (EBOV) and Sudan (SUDV), and contains internal extraction and sample controls. The prototype Ebola Virus triage test was evaluated at I™ starting from 200 µL EDTA-whole blood samples spiked with EBOV and SUDV nucleic acid material. Results were compared with the in-house reference real-time RT-PCR and the RealStar® Filovirus Screen RT-PCR kit (Altona diagnostics). The preliminary data demonstrated that the Idylla™ prototype Ebola virus triage test is a promising tool for near-patient EVD testing. The test is fast, safe and easy-to-use.

Oral abstracts

Abstract number : 50

Comparison of diagnostic and bioinformatics real-time PCR assays for detection of human astroviruses

RHT Nijhuis, I.A. Sidorov, A.A. Gulyaeva, A.E. Gorbalenya, E.C.J. Claas
LUMC, LEIDEN, Nederland

In the past decennium, numerous real-time PCR assays have been developed for the detection of microorganisms. The development of such assays can be challenging, especially for RNA-viruses with highly variable genome sequences. Because of the advancing knowledge about the RNA-virus diversity, regular evaluation of diagnostic oligonucleotides is mandatory. In this study, we compare the quality of our diagnostic human astrovirus (HAsV) primer-probe mix (PPM) and a so-called bioinformatics HAsV PPM, which was designed using a recently developed bioinformatics pipeline for PCR evaluation and design *in silico* (Sidorov & Gorbalenya, in preparation).
Based on a multiple sequence alignment of all publicly available genomes and phylogeny of the *Astroviridae* family and the required PCR design parameters, the pipeline selected the best possible PPM out of the more than 30,000 calculated. This bioinformatics PPM was located in the orf1b genome region and had the predicted sensitivity of 99.1%, whereas the predicted sensitivity of our diagnostic PPM (orf1a genome region) was calculated to be 85.9%. Both PPMs were shown about 100% selective. These PPMs were subsequently compared in real-time PCR using a panel of eight cultured HAsV isolates (types 1-8). Both PPMs detected all HAstV isolates, although the diagnostic PPM showed a Ct-value of 4 and 7 higher for AsV type 6 and type 7 respectively, compared to the bioinformatics PPM.
In conclusion, the pipeline calculated a bioinformatics PPM that showed a higher sensitivity compared to our diagnostic PPM *in silico*, what could be confirmed by testing a variety of cultured HAstV isolates in real-time PCR. Although these results already indicate an improvement in detecting HAsV, the added value of the bioinformatics PPM needs to be established also in samples that were tested negative in our diagnostic assay.

Oral abstracts

Abstract number : 55

Clinical metagenomics: a new paradigm for the diagnosis of infectious diseases?

ER RUPPE[1], DB Baud[1], SC Schicklin[2], ES Santiago-Allexant[2], GG Guigon[2], JS Schrenzel[1]
[1]HOPITAUX UNIVERSITAIRES DE GENEVE, GENEVE, Switzerland
[2]BioMérieux, MARCY L'ETOILE, France

Metagenomics, based on next-generation sequencing (NGS), has been available for a decade. In infectious diseases, genomics has been showed to be a promising tool in terms of identification, typing and antibiotic susceptibility testing (AST) of pathogens, but the proof of concept for metagenomics (direct sample sequencing) has to be done. While the turnover of NGS is still slower than conventional culture, it is expected in a near future to be reduced enough to become compatible with a fast diagnosis of infectious diseases (that we refer as clinical metagenomics).
Indeed, new solutions are expected as the steadily increasing burden of multidrug-resistant (MDR) bacteria compromises the optimal management of several infections. In many cases, the unexpected presence of a resistant bacteria as a causal agent for an infection causes delay in providing the patient with an adequate antibiotic treatment. In severe infections such as hospital-acquired, ventilatory-associated or healthcare-associated pneumonia (HAP, VAP and HCAP, respectively), this delay can have major clinical consequences since the later an adequate antibiotic regimen is given, the worse the prognosis (more mortality, more morbidity, longer hospital stay). This challenge is currently not addressed by conventional microbiology techniques that require at least 48 hours to provide an antibiotic susceptibility profile of the causing agent(s).
Even if NGS could soon become faster than culture, many obstacles remain to be overcome before clinical metagenomics could be used as a routine diagnostic tool, namely the choice of antibiotic resistance determinants (ARDs) to consider, the quantification of pathogens, the inference of an antibiotic susceptibility profiles from metagenomic data and the linkage between ARDs and their host.
In this presentation, we will provide a bottom-up, clinically-driven framework for the application of clinical metagenomics in HAP/VAP/HCAP and show through specific examples the benefits that could be expected.

Oral abstracts

Abstract number : 57

Differentiation between second primary lung cancers and metastatic disease using next-generation sequencing

P Roepman[1], A Heuvel, ten[2], K Scheidel-Jacobse[1], DAM Heideman[3], GJM Herder[2], JA Kummer[1]
[1]Pathologie DNA, NIEUWEGEIN, Nederland
[2]St Antonius Hospital, NIEUWEGEIN, Nederland
[3]VUmc, AMSTERDAM, Nederland

Introduction
Lung cancer may present with multiple synchronous or metachronous lesions. Differentiation between multiple primary lung cancers (MPLC) or metastatic disease (PM) has important prognostic and clinical implications. Current techniques for diagnosing multiple lung cancers (MLC) are laborious and require substantial amounts of material. Next generation sequencing (NGS) may be a more accurate alternative, providing a comprehensive mutational profile using only a small amount of tissue. NGS also provides information on mutations in important oncogenes.
Study setup
We analyzed archived tissue from fifty patients with MLC treated surgically in the St. Antonius Hospital between 2003 and 2014. Original diagnosis of MLC was based on ACCP criteria, histology, immuno-histochemistry, and classical p53 Sanger sequencing. We compared diagnosis of MLC with targeted resequencing by NGS (using the Ion Torrent Cancer Hotspot panel) to classical p53 analysis.
Results
On the first batch of 31 tumours from 15 patients, NGS showed a technical success rate of 100%. On average, we identified 2-3 hotspot mutations per patient in 17 different genes. Most mutations were found in TP53, followed by KRAS, EGFR, and CDKN2A and FBXW7. Eight patients showed different mutation profiles between tumours and were diagnosed as MPLC; three patients shared identical mutation profiles (diagnosed PM). In two patients mutational profiles suggested MPLC, but progression could not be excluded and in two patients analysis of normal tissue is required for a definite diagnosis (ongoing). Analysis by NGS will eventually be conclusive in 13/15 patients (87%). Classical p53 Sanger sequencing resulted in only 46% of patients in a diagnosis.
Conclusion
Targeted resequencing using NGS provides a more accurate and comprehensive diagnosis of multiple lung cancers than classical p53 mutation analysis.

Oral abstracts

Abstract number : 58

Improvement of diagnostic method validation processes in a Finnish hospital laboratory using lean methods and a software solution

T Hirvonen[1], A Virtanen[1], L Mannonen[2], R Loginov[2], M Lappalainen[2]
[1]Finbiosoft, ESPOO, Finland
[2]HUSLAB, HELSINKI, Finland

Background
Developing new in-house assays or verifying commercial kits in hospital laboratories is increasingly challenging due to strictening regulatory and accreditation requirements. For example, ISO 15189 and the upcoming CE-IVD set new requirements for validations and verifications. These projects are already complex and burdening, involving a lot of error-prone manual work mainly done in Excel sheets and paper documents.
Objectives
This study aimed to eliminate manual data management (waste) and improve the quality of validations and verifications using lean methods and a novel validation software to standardize and automate the validation and verification processes.
Case description
This study was done in cooperation between HUSLAB, the largest university hospital laboratory in Finland with nearly 20 million laboratory tests conducted annually, and Finbiosoft, a Finnish software company developing Validation Manager™ software. The study focused on validation and verification projects conducted at HUSLAB department of virology and immunology. The studied validation processes were analyzed using lean methods, and an improved process model was developed to replace earlier practices. To support the new lean model, Validation Manager™ was developed further to standardize and automatize the validation processes in clinical laboratories.
The new process and validation software were evaluated in three validation projects. The evaluation implied that up to 95% of manual data management time could be saved. The standardized process and automated software tool were also found to reduce errors and to improve several quality aspects, including result reliability and traceability, and process transparency.
Conclusions
The case study implies that the new lean process together with the validation software enable hospital laboratories to conduct validation studies more efficiently, with increased quality and according to latest regulatory requirements.

Oral abstracts

Abstract number : 66

Multicenter performance evaluation of BRCA MASTR™, CFTR MASTR™ and FMF MASTR™ on Roche 454™ and Illumina MiSeq® massively parallel sequencing platforms

JDF Del Favero
Multiplicom N.V., NIEL, Belgium

Noor Remmerie[1,2], Sarah Berwouts[1,2], Martine Goergen[1], Jurgen Del-Favero[1]

Massively parallel sequencing (MPS) technologies are providing a foundation on which targeted enrichment methods or targeted amplification methods such as multiplex PCR-based technologies can be applied to detect congenital genetic defects or to determine whether an individual has an increased genetic risk to develop a disease.
A multicenter performance evaluation study was carried out of three MASTR (Multiplex Amplification of Specific Targets for Resequencing) kits, enabling the amplification and detection of germline variants associated with breast cancer (BRCA MASTR™), cystic fibrosis (CF, CFTR MASTR™) and familial Mediterranean fever (FMF, FMF MASTR™). These studies were performed in support of a European (EU) *in vitro* diagnostic (IVD) regulatory submission.
Sensitivity, specificity, accuracy and reproducibility were assessed for each kit, using 146 breast cancer, 187 CF and 124 FMF DNA samples, selected by eight genetic testing laboratories from five European countries. Amplicon libraries were sequenced on Roche 454™ or Illumina MiSeq® platform. Sequence reads were mapped to a reference sequence and variants analysed using Sequence Pilot© and Sophia Genetics DDM platform.
We will show and discuss (i) how the multicenter generated MPS data can be used to comply with EU regulatory IVD criteria and (ii) how to warrant the quality of the diagnostic kit for the end user. Also, we will discuss the consequences and challenges that EU genetic diagnostic centers have to face in the coming years based on the fast evolving MPS based genetic analysis.

Oral abstracts

Abstract number : 67

Five years of experience with ISO 15189 accreditation in Belgian molecular diagnostics laboratories. Presented on behalf of MolecularDiagnostics.be

M Raymaekers[1], L Cnops[2], B Vanmassenhove[3], J Gras[4], K Vandepoele[5], A Vankeerberghen[6], F Vaeyens[6], P Descheemaeker[7], P Van Lint[8]
[1]Jessa Hospital, HASSELT, Belgium
[2]Institute of Tropical Medicine, ANTWERP, Belgium
[3]AZ Damiaan, OSTEND, Belgium
[4]St. Luc Bouge Hospital, NAMUR, Belgium
[5]Ghent University Hospital, GHENT, Belgium
[6]OLVZ-Aalst, AALST, Belgium
[7]AZ Sint-Jan Brugge-Oostende AV, BRUGES, Belgium
[8]GZA St-Augustinus, WILRIJK, Belgium

Since 2007, International Organization for Standardization (ISO) 15189 accreditation has been required for Belgian molecular diagnostic laboratories to be reimbursed for performing diagnostic tests. In response, the MolecularDiagnostics.be working group (MD.be) was founded to discuss the practical implementation of ISO 15189 for accreditation. This resulted in a publication offering practical guidelines to laboratories wanting to achieve ISO 15189 accreditation. The 2012 update of ISO 15189 provided an excellent opportunity to review and evaluate the non-conformities received by MD.be members during past external audits. Therefore, MD.be organized a survey of Belgian molecular laboratories and this showed that many laboratories received similar audit non-conformities during the last five years. Furthermore, a gap analysis between ISO 15189:2007 and ISO 15189:2012 revealed some new requirements demanding changes in laboratory procedures. We hereby discuss the 421 non-conformities received in view of the new ISO 15189:2012 requirements. By highlighting topics which require special attention, this study can help molecular diagnostic laboratories prepare for ISO 15189:2012 accreditation.

Oral abstracts

Abstract number : 90

Analysis of volatile metabolites for disease diagnosis, phenotyping and prognosis.

M.P van der Schee
University of Amsterdam, Amsterdam, Nederland

Medical diagnosis and phenotyping increasingly incorporate information from complex biological samples. This has promoted the development and clinical application of non-invasive metabolomics in exhaled air (breathomics), stool and urine. In respiratory medicine, expired volatile organic compounds (VOCs) are associated with inflammatory, oxidative, microbial and neoplastic processes. After recent proof of concept studies demonstrating moderate to good diagnostic accuracies, the latest efforts in VOC studies are focused on optimization of sensor technologies, analytical algorithms and independent validation of clinical classification and prediction. Current research strategies are revealing the underlying pathophysiological pathways as well as clinically acceptable levels of diagnostic accuracy. Implementing recent guidelines on validating molecular signatures in medicine will enhance the clinical potential of volatile metabolomics and its development as a point-of-care technology.

POSTER ABSTRACTS BY ORDER OF ABSTRACT NUMBER

Poster abstracts

Abstract number: 1

Hydrogel microarray platform for in vitro allergy diagnostics

O S Smoldovskaya, G F Feyzkhanova, S V Voloshin, A A Arefieva, A Z Zasedatelev, A R Rubina
Engelhardt Institute of Molecular Biology Russian Academy of Sciences, MOSCOW, Russian Federation

Number of people suffering from allergy increases from year to year. That is why useful and accurate tests for allergy diagnostics are very necessary. *In vivo* methods (provocation and skin tests) used for diagnostics of allergy type I disease may result in acute reaction to allergen intake, so they are replaced by *in vitro* tests. *In vitro* tests enable to measure the concentration of specific immunoglobulins class E (sIgE), which are responsible for hypersensitivity reaction, in serum samples, as well as the concentration of specific immunoglobulins class G4 (sIgG4), presumably decreasing allergic response operating as blocking antibodies. Multiplex test-system Allergochip, developed in EIMB RAS, enables to carry out simultaneous quantitative analysis of sIgE and sIgG4 panel to most common allergens and their recombinant proteins. The test-system is based on the antigen-antibody interaction and represents modification of solid phase immunochemical analysis with fluorescent signal registration in biochip format. Allergochip includes 2 identical clasters of 0,1 nl hydrogel spots with immobilized molecular probes: allergen extracts and recombinant proteins.
During the incubation of serum sample on allergochip sIgE and sIgG4 bind with their specific antigens. Antigen-antibody complex visualization was perfomed by anti-human antibodies conjugated with fluorescence dye Cy5. For one claster anti-human IgE-Cy5 antibodies were used, for another -anti-IgG4-Cy5 antibodies. The fluorescence signal from the triple complex was detected by Biochip Analyzer, developed in EIMB RAS. Calculation of allergen-specific antibodies concentration (sIgE and sIgG4) was derived from the calibration curve by special software ImageAssay.
Comparison of the results obtained using our diagnostic test-system and multiplex test system MAST-CLA (Hitachi, USA) confirms good quality of Allergochip (AUC in ROC-analysis for inhalant allergens 0,82, for food allergens 0,78). Data received show promising outlook of biochip application for allergy diagnostics in the near future.

Poster abstracts

Abstract number: 2

Evaluation of a Rapid Molecular Method to Detect Mycobacterium tuberculosis using the BD-MAX system in a European Multicenter Study.

I.O.M. Op den Buijs[1], A.L. Seagar[2], P. Baltar[3], R.T.J.M. Roymans[1], K.E. Templeton[2], I.F. Laurenson[2], G. Barbeito[3], B. Regueiro[3], J.H.B. Van de Bovenkamp[1]
[1]Stichting PAMM, VELDHOVEN, Nederland
[2]Scottish Mycobacteria Reference Laboratory, EDINBURGH, United Kingdom
[3]Clinical Hospital of Santiago, SANTIAGO DE COMPOSTELLA, Spain

Background: Tuberculosis (TB), caused by *Mycobacterium tuberculosis*(MTB),is a common cause of death. Rapid laboratory diagnosis is vital in limiting spread of infection. Smear microscopy is used to screen for Mycobacteria but does not differentiate species, is laborious and requires trained personnel. Direct detection of MTB using PCR based assays provides a fast, sensitive and specific alternative. The purpose of this study was to evaluate qPCR for MTB detection using the automated BD-MAX system. Custom made snap-in tubes, containing dried primers and probes, were evaluated at three European laboratory sites. Methods for all sites:-QCMD MTB2012 quality control panel was tested.-Retrospective study: 275 samples processed by the standard protocols adopted at each laboratory site.-Prospective study: 299 samples were processed by BBL MycoPrep. qPCR results were compared to smear microscopy and liquid culture results using MGIT960. True positivity was defined as a specimen obtained from a patient who developed active TB confirmed by culture.**Results:**-Quality control: a 100% score was achieved for MTB2012.-Retrospective: 191 true-negative and 84 true-positive samples were determined by study parameters. Three true-positive (smear negative) samples were not detected by qPCR. Sensitivity, specificity, positive and negative predictive value were 96.4%, 100%, 100% and 98.5%, respectively.-Prospective: 227 true-negative and 72 true-positive samples were determined by study parameters. Eleven smear-negative samples showed false-negative qPCR results. Sensitivity, specificity, positive and negative predictive values were 84.7%, 100%, 100% and 95.4%, respectively. The prospective evaluation included more smear-negative samples from patients with active TB. Finally, 100% correlation was found between qPCR and MTB smear positive results.**Conclusions:**qPCR on the automated BD-MAX in combination with the utilization of dried primer-probe tubes is a rapid, simple, sensitive and specific method for MTB detection. This methodology was 100% sensitive and specific for smear positive samples. Utilization of this test could change laboratory practice by providing a rapid method for MTB screening.

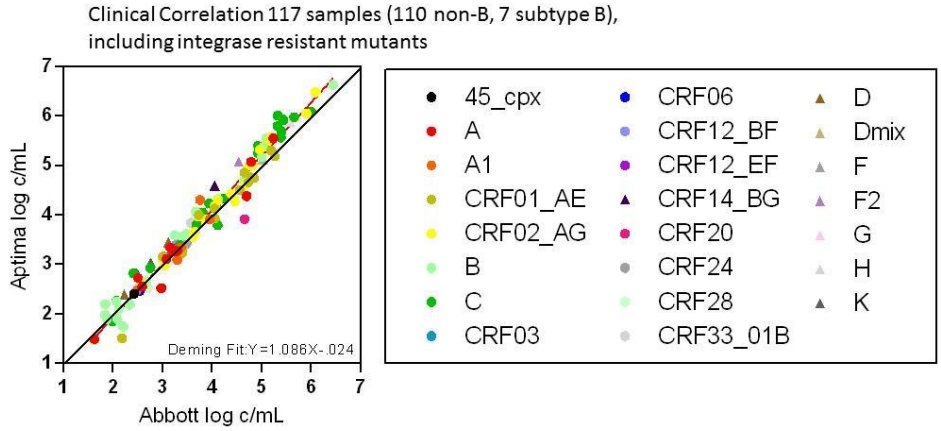
Poster abstracts

Abstract number: 3

Hologic Aptima HIV-1 Quant Dx Assay Compared to Abbott RealTime in B-/Non-B-Subtypes in daily routine testing

R Ehret, SM Schuetze, AM Moritz, MJO Obermeier
MIB, BERLIN, Germany

Background: Hologic's Aptima HIV-1 Quant Dx is a HIV-1 RNA quantitative assay based on real-time Transcription-Mediated-Amplification (TMA) that runs on the fully automated Panther system with random access. A comparison with the Abbott m2000 RealTime assay was performed. Special focus was put on viremia near the limit of detection, non-B subtypes and integrase inhibitor resistant samples.
Methods: Fresh (n=282), frozen (non-B, n=117; with integrasemutations, n=22) and diluted (n=559) patient samples were tested. The Aptima assay uses dual target execution (probes in integrase and LTR), whereas RealTime uses one probe (integrase). Samples with integrase resistance-mutations were additionally tested with Roche Cobas TaqMan v2.0 using probes with different target regions. Hand-on time and time to results were evaluated in routine diagnostic testing.
Results: With a LLOQ of 30cps/mL LLOD of 13cps/mL, the Aptima assay classified more samples as "detected"(30 versus 6) than the RealTime assay in 100 unselected fresh samples. High concordance was also shown for non-B subtypes. In Bland Altman plots the mean difference was below 0.1log_cps/mL. Intra- and inter-assay variation was low and comparable to RealTime with intraassay %CV ranging from 4.0% (2.0log_cps/mL) to 8.4% (1.7log_cps/mL). Linearity was shown by serial dilution (subtypes B, C and CRF02_AG) from 5.7 log_cps/mL to 1.7 log_cps/mL; the slopes ranged from 0.99 to 1.04. Resistance-mutations in the integrase were not found to impact results. Performance of Aptima in high throughput, routine use was excellent with lower hand-on time and faster result achievement compared to RealTime.
Conclusions: The Aptima HIV-1 Quant Dx assay showed excellent correlation with RealTime with high sensitivity, linearity and accuracy in the therapeutic relevant range for all tested HIV-1 subtypes. With random access, the ability to continuously load samples and time to first result of 153 minutes it is a major improvement in the viral load monitoring of HIV-1 infection.



Poster abstracts

Abstract number: 5

Comparison of six commercial kits for pathogen DNA extraction from whole blood

MC Cernochová, HO Obrucová, EN Nemcová, MV Vanerková, TF Freiberger
Centre for Cardiovascular Surgery and Transplantation, BRNO, Czech Republic

Objectives: Sensitive pathogen detection from whole blood still remains an unresolved issue. Efficient DNA extraction is crucial for subsequent fast molecular assays. Here we present a comparison of six commercially available DNA extraction kits for whole blood.
Methods: Freshly cultured *Staphylococcus aureus* and *Klebsiella pneumoniae* in brain-heart infusion were serially diluted and spiked into fresh EDTA whole blood to final concentrations 10^5 - 10^2 CFU/ml. DNA from blood was extracted using MolYsis Complete5 (Molzym, Germany), RTP Bacteria DNA Mini Kit (Stratec, Germany), Invisorb Spin Blood Midi Kit (Stratec, Germany), QIAamp UCP Pathogen Mini Kit (Qiagen, Germany) with mechanical pretreatment, innuPREP Blood DNA MIDI Direct Kit in combination with LOOXSTER Enrichment Kit (Analytik Jena, Germany) and QIAamp DNA Blood Mini Kit (Qiagen, Germany) with freeze-thaw and enzymatic pretreatment. The endpoint PCR was performed using universal eubacterial primers targeting the whole 16S rRNA gene.
Results: QIAamp UCP Pathogen Mini Kit with DNA extraction from 400 ul blood showed the highest sensitivity with detection limit 10^2 - 10^3 CFU/ml for *S. aureus* and 10^2 CFU/ml for *K. pneumoniae*. Kits differed in blood volume used for DNA extraction or elution volume which could have influenced final sensitivity. Two kits enabled selective pathogen DNA enrichment and one kit included optional mechanical pretreatment. Additional parameters like price or hands-on-time were also considered.
Conclusion: Our results showed the extraction by QIAamp UCP Pathogen Mini Kit as the most efficient and sensitive commercially-available method. Extracted DNA had sufficient length and quality enabling the amplification of the whole 16S rRNA gene, which allowed subsequent pathogen identification by sequencing or hybridisation.
The study was supported by Hutman Diagnostics AG (Basel, Switzerland) and by the grant CKTCH No. 201402.

Poster abstracts

Abstract number: 6

Prevalence and disease burden of Trichomonas vaginalis and Mycoplasma genitalium in men attending a Sexually Transmitted Infections clinic in Amsterdam, the Netherlands

S.M. Bruisten, C. van der Veer, M.S. van Rooijen, M. Himschoot, HJC de Vries
GGD Amsterdam, AMSTERDAM, Netherlands

Introduction:
Men are not routinely tested for *Trichomonas vaginalis* (TV) and *Mycoplasma genitalium* (MG) in the Netherlands and, therefore their prevalence and/or role in urogenital complaints in the Dutch male population is unknown. Our aim was to describe the age-specific prevalence of TV and MG and the possible association of TV and MG infections with male urogenital complaints, ethnicity, high-risk sexual behavior and co-infections with *Chlamydia trachomatis* (CT), in men attending the Sexually Transmitted Infection (STI) clinic in Amsterdam, the Netherlands.
Methods:
Urine samples and clinical data were collected from 526 heterosexual men and 678 men who have sex with men (MSM) attending the STI clinic. To investigate age as a risk factor, we oversampled older men. Urine samples were tested for TV and MG using TMA (Hologic) for TV and CT and an in house PCR for MG.
Results:
TV infection was rare in heterosexual men (n=6; 1.1%) and non-existent in MSM attending the STI clinic. TV cases were mostly older than 40, of non-Dutch ethnicity and associated with low-risk sexual behavior. MG infection was equally common in both MSM and heterosexual men (3.1%). No age or ethnic trends were observed for MG infection, however, high-risk sexual behavior in MSM correlated with MG infection. Co-infections of TV or MG with CT were rare (< 0.5%). Of the patients reporting urogenital symptoms 5.9% were positive for MG, including one TV/MG co-infection.
Conclusion:
TV infection is rare and asymptomatic among men attending the STI clinic in Amsterdam. MG is quite common in men, but also remains mostly asymptomatic. While the outcome of this study does not encourage general testing for TV in men, it does, however, suggest that some male urogenital symptoms - not caused by gonorrhoea or CT- could be explained by MG infection.

Poster abstracts

Abstract number: 7

A duplex real-time TaqMan PCR assay for the rapid and reliable detection of allele HLA-B*27 in the Caucasian population.

FV Vaeyens[1], E Bailleul[2], P Meeus[2], C Indevuyst[3], A Vankeerberghen[2], H De Beenhouwer[2]
, AALST, Belgium
[2]OLV Hospital, AALST, Belgium
[3]MCH, LEUVEN, Belgium

Objectives: The HLA-B*27 genotype is strongly associated with spondyloarthropathies, a group of inflammatory rheumatic diseases including ankylosing spondylitis. New HLA-B*27 subtypes are constantly discovered. Our objective was to develop and validate a real-time PCR for HLA-B*27 which can detect all known and clinically significant HLA-B*27 genotypes present in the Caucasian population. The assay, targeting HLA-B*27 and β globin as an internal control, was intended to function on a fast real-time PCR platform. Methods: Primers and probes described in literature were selected and adjusted to specifically target all known and clinically relevant Caucasian HLA-B*27 alleles. Because HLA-B*27 negative samples show a limited cross-reaction with the HLA-B*27 PCR, an interpretation algorithm of the amplification data was generated based on delta Ct (HLA-B*27 Ct - β globin Ct) cut-off values and a maximal acceptable β globin Ct. The accuracy was assessed on 69 clinical samples and 30 EQC samples with the traditional PCR as a reference method. The validity of the interpretation algorithm was tested and the intra-run and inter-run precision were determined. Results: For the EQC samples (16 HLA-B*27 positives and 14 negatives) complete concordance was found between the duplex PCR and the conventional method. Traditional PCR results of the clinical samples (19 HLA-B*27 positives and 50 negatives) were confirmed by the duplex PCR. The maximal intra-run and inter-run variation was less than respectively 0.22 sd (mean Ct 23.44) and 0.66 sd (mean Ct 22.93). The validity of the interpretation algorithm was accepted. Conclusions: This duplex real-time PCR is suitable for rapid and reliable HLA-B*27 genotyping in the Caucasian population.

Poster abstracts

Abstract number: 8

Multi-omics diagnostics for tailoring treatment in respiratory tract infections and sepsis.

J Hays
Erasmus MC, ROTTERDAM, Netherlands

Rapid diagnosis will help reduce the inappropriate use of antibiotics, one of the main reasons for global increases in antibiotic resistance. One option for rapid diagnosis is the use of -omics technologies.
The 'TAILORED-Treatment' (www.tailored-treatment.eu) consortium is an EU FP7 funded project comprising 7 European partners. Our main goal is to establish an omics-based diagnostic strategy that can be implemented to increase the effectiveness of antibiotic and antifungal therapy, reduce adverse events, and help limit the emergence of antimicrobial resistance in children and adults.
At the heart of the TAILORED-Treatment project is a prospective clinical study in which we will recruit 1200 patients (>2000 patient samples) presenting with respiratory tract infections and/or sepsis. Patient cohorts will include equal representation of genders, children and adults.
State-of-the-art molecular and biochemical technologies (transcriptomics, proteomics, genomics, microbiota analysis) will be applied to characterize the host-pathogen response. The data collected will be added to a large-scale unique multi-dimensional dataset which will be stored in a publically available database, and will be accessible to the EU scientific and clinical community.
We will construct a predictive personalized treatment algorithm that will lead to informed and personalized antibacterial, antifungal and antimicrobial treatment regimens (indication, dosage, and duration) that are tailored to the needs (type of infection, presence of novel biomarkers etc) of children and adults presenting with respiratory infections and sepsis.
The algorithm and large-scale unique multi-dimensional dataset will be built into an easily navigable web-based, free-to-use, decision support system ready for use by physicians to explore, test and assist in patient-tailored antimicrobial treatment decisions.
Ethical approvals have been granted and clinical samples are being collected and processed. Preliminary results are expected to be available in 2016.

Poster abstracts

Abstract number: 10

Development of an internal process control (IPC) and analysis of PCR-artefacts

E. van Pelt-Verkuil, W.B. van Leeuwen, L Coenen
Professional university Hogeschool Leiden, LEIDEN, Nederland

An IPC (MS2-phage) was developed for RT-qPCR of pathogenic RNA viruses for 1st line control in routine molecular diagnostics in shellfish and quantitative analysis of analytical steps during set up of in house assays. MS2 specific primers/probes were selected with AlleleID. Amplicons were checked by melting curve analysis (MCA), agarose gel-electrophoresis, sequencing and LinRegPCR and compared with a published set[1] for sensitivity/efficiency, amplification characteristics and aberrant melting peaks. LOD was established with intercalating dye (Eva Green; EG) and probe (Cy5) assays.
Three primersets were different with respect to PCR-efficiency/specificity; the published primerset was the worst performer. Non-specific products (NSP) were synthesised at the same efficiency as MS2 amplicons. Although NSP are not detected in probe assays, their presence interferes with virus detection at LLOD. Exclusion of these samples resulted in comparable LLOD's for intercalating dye and probe assays indicating the amount of detectable MS2 virus particles at about 1.
Best primerset was used for modelstudies of analytical steps. Considerable loss (10^4X) of targets occurred in tissue matrix and proteinase K incubation at 37 ˚C. Isolation with MiniMag or cDNA synthesis was less critical, although overload of the magnetic beads during nucleic acid isolation correlated with the formation of non-specific products at high dilutions.
Conclusions:
Probe assays don't detect NSPs but when present, they interfere with efficiency. Primer checks with intercalating dyes, also from published ones, are essential
"Inhibition"is not confined to qPCR itself; target loss due to analytical procedures is more important
Loss of microbial targets during analytical steps cannot be calculated exactly, however, our MS2 matrix-model, points to lower LLOD than expected.
[1] doi:10.1128/JCM.43.9.4551-4557.2005

Poster abstracts

Abstract number: 11

Rapid antimicrobial susceptibility testing in bacteremic patients with Raman spectroscopy

C.C. Orelio[1], H.E. Dekter[1], M.C. Morsink[1], S Tektas[1], B. Vis[1], R te Witt[2], W.B. van Leeuwen[1]
[1]Hogeschool Leiden, LEIDEN, Nederland
[2]Erasmus Medical Center, ROTTERDAM, Nederland

H.E.Dekter[1], C.C.Orelio1, M.C.Morsink[1], S.Tektas[1], B.Vis[1], R. te Witt[2], W.B. van Leeuwen[1,2]
1. Innovative Molecular Diagnostics, University of Applied Sciences Leiden, The Netherlands
2. Medical Microbiology and Infectious Diseases, Erasmus Medical Centre Rotterdam, The Netherlands

Bacteremic patients are prone to sepsis, which can result in rapid deterioration of the patient's health. Currently, bacterial identification and antimicrobial susceptibility testing (AST) takes 60-80 hours. Here, we investigated the performance of Raman spectroscopy for AST in bacterial cultures and bacteria isolated from blood culture bottles. In total, 66 antibiotic resistant and 68 antibiotic sensitive bacterial strains were incubated for five hours in the presence or absence of antibiotics. Bacteria were analysed with Raman spectroscopy to determine antimicrobial susceptibility. Thirteen bacterial strains isolated from blood cultures were analysed in a similar manner. All Raman spectral analyses were compared to Vitek®2 results.
Our results showed that antibiotic-sensitive bacteria cultured in the presence of antibiotics display discriminative differences in the Raman spectrum as compared to cultures in the absence of antibiotics. Antibiotic resistant bacteria did not reveal such differences. The changes in Raman spectra corresponded with previously determined MIC by Vitek®2 technology in 83% of analysed bacterial strains. Bacterial strains isolated from blood and processed in a similar manner revealed that Raman spectroscopic analysis have a concordance of 100% with Vitek®2 results.
In conclusion, Raman spectroscopy is able to detect bacterial antimicrobial susceptibility within five hours after bacterial culture or positive blood culture.
Comparison of Raman spectroscopy to Vitek®2 method showed that Raman spectroscopy was robust and reliable, thereby providing proof-of-concept for AST with Raman spectroscopy. Importantly, an AST profile of bacteria isolated directly from positive blood cultures can be produced within seven hours, thereby shortening the period-to-diagnosis with 16-20 hours as compared to Vitek®2 analysis.

Poster abstracts

Abstract number: 12

NGS pilot study of E coli ESBL from patients with suspected sepsis

HE Enroth[1], H Wefer[2], L Ljungström[3], D Tilevik[4]
[1]Unilabs AB, SKÖVDE, Sweden
[2]SciLife Lab, SOLNA, Sweden
[3]Skaraborg Hospital, SKÖVDE, Sweden
[4]University of Skövde, SKÖVDE, Sweden

Helena Enroth[1], Hugo Wefer[2], Lars Ljungström[3], Diana Tilevik[4].
[1]Unilabs Skövde, [2]SciLife Lab Solna, [3]Skaraborg Hospital Skövde, [4]University of Skövde, Sweden
The “Sepsisstudy Skaraborg”was performed during nine months 2011-2012 and included patients with suspected sepsis. From these patients, 2,300 pathogenic bacterial isolates, from different sample types and locations, were collected. *Escherichia coli* was the most common finding, with about 500 isolates, of which a few were classified as ESBL. The aim of our pilot study was to compare *E coli* ESBL and *E coli* isolates from patients with sepsis or severe sepsis. A secondary aim was to acquire knowledge of the workflow for NGS methodology as well as bioinformatic analysis of such data for bacterial genomes.
Twenty-one *E. coli* isolates (9 ESBL) from 16 patients were cultured and DNA was extracted on Roche MPCompact according to conventional methods. DNA was transported to SciLifeLab in Solna, Sweden, for Nextera XT library preparation and sequencing on the Illumina MiSeq system. Duplicate sequences were removed using FastUniq and the remaining sequences were processed with SeqPrep, for adaptor removal and quality trimming. Genomic assembly was performed twice, using SPAdes and RAY respectively. Analysis of the assembled contigs for each isolate was performed using free web-based tools available at the Center for Genomic Epidemiology website.
Each sample yielded on average 626 contigs with a length of over 500 bases, with the longest contigs at around 94,000 basepairs. Pairs of isolates from the same patient, but different locations, gave almost identical results for plasmids, resistance- and virulence genes, and MLST sequence type. The four most common virulence genes corresponded well with uropathogenic *E. coli* (UPEC). Most virulence- and resistance genes were located on the plasmids found in the *E. coli* ESBL isolates.

Poster abstracts

Abstract number: 14

Single cell MALDI-TOF based typing of Serratia marsescens strains obtained from hospitalized patients.

H.E. Dekter[1], R.R. Parchen[2], C.C Orelio[1], F. Koyuncu[2], A. van der Zee[3], G.A. Parlevliet[4], W.B. van Leeuwen[1]
[1]University of Applied Sciences, LEIDEN, Nederland
[2]BiosparQ, LEIDEN, Nederland
[3]Department of Medical Microbiology and Infectious Diseases Maasstad hospital, ROTTERDAM, Nederland
[4]Department of Medical Microbiology Bronovo hospital, DEN HAAG, Nederland

Single cell MALDI-TOF is a new platform capable of presenting bacterial cells individually to the ionization unit, with each cell producing a classifiable mass spectrum, enabling a quantitative analysis of a sample even if this contains a mix of microorganisms.
In this study we compared two typing methods, AFLP and SC-MALDI-TOF, to determine whether *Serratia marcescens* strains, collected from hospitalized patients are identical and whether the rapid SC-MALDI-TOF technique was able to type the strains as accurate, compared to the molecular technique AFLP.
Ten *S. marsescens* strains were cultured on bloodagar plates for AFLP typing. For SC-MALDI-TOF analysis, strains were cultured in TSB. Bacterial pellets were prepared by washing and centrifugation steps and finally resuspended in matrix for SC-MALDI-TOF. This mixture was subjected to the SC-MALDI-TOF.
After analysis, the AFLP data showed two identical strains (similarity >90%). The similarity among the other 8 strains were below the 90% and thus considered unique.The clustering results of the SC-MALDI-TOF were similar to the results of AFLP.
The reference method, AFLP, and the SC-MALDI-TOF were fully concordant for S. marsescens. This is the first experiment that indicates that SC-MALDI-TOF is an accurate and rapid method for typing of S. marsescens.

Poster abstracts

Abstract number: 16

An investigation into DNA recovery and stability of five common enteric bacteria using the MWE Fecal Transwab® as a transport device

FK Kartal, A Rossouw, VLA James
Public Health England, LONDON, United Kingdom

UK NEQAS for Microbiology is an external quality assessment (EQA) provider with participating laboratories worldwide. EQA is an invaluable tool for clinical laboratories monitoring the performance, quality and reliability of their service. UK NEQAS for Microbiology sought to determine if Fecal Transwab® (Medical Wire & Equipment) provided a suitable sample delivery device for an EQA scheme targeting enteric bacteria detection. The five common enteric pathogens included: *Clostridium difficile*, *Campylobacter jejuni*, *Shigella sonnei*, *Salmonella typhimurium* and *Yersinia enterocolitica.*
Duplicate Fecal Transwab were inoculated with a 100µL suspension containing one of the five defined bacteria considered and stored at both 4°C and 22°C over a period of 112 days. DNA recovery was initially assessed at the start of inoculation, day 1 and day 2, thereafter a weekly assessment was performed over a period of a month, followed by a monthly assessment for 3 months, totaling a 4 month period (112 days). The detection of the enteric pathogens was achieved using the Fast-Track Diagnostics Bacterial gastroenteritis multiplex PCR kit. All pathogens remained detectable throughout the study period: whether stored at 4°C or 22°C, showing no significant increase or decrease in the bacterial load detected.
The study results suggest that the Fecal Transwab® could be a suitable sample format for use in developing an EQA scheme for molecular detection of enteric pathogens. In current literature, DNA recovery and stability studies from transport media and swabs are limited; hence this investigation has introduced new insight into this field.

Poster abstracts

Abstract number: 18

GC heterozygotic status at IL6 -174 G>C SNP and increased risk of congenital infection with Toxoplasma gondii

W.I. Wujcicka, Z.Z. Gaj, J.Sz. Wilczynski, D.E. Nowakowska
Polish Mother's Memorial Hospital - Research Institute, LODZ, Poland

Wujcicka W[1,2], Gaj Z[1,2], Wilczyński J[2], Nowakowska D[2]
Scientific Laboratory of Center of Medical Laboratory Diagnostics, Polish Mother's Memorial Hospital - Research Institute, Lodz, Poland
Department of Perinatology and Gynecology, Polish Mother's Memorial Hospital - Research Institute, Lodz, Poland
e-mail: wujcickaw@wp.pl

Purpose Estimation of distribution of distinct genotypes and alleles located at interleukin 6 (*IL6*) and *IL1* single nucleotide polymorphisms (SNPs), between fetuses and neonates with and without congenital *Toxoplasma gondii* infection.
Methods Research included 22 fetuses and newborns infected with *T. gondii* and 49 uninfected offsprings. The levels of IgG and IgM antibodies against parasite, and IgG avidity, were estimated using ELFA tests. *T. gondii* DNA loads in amniotic and cerebrospinal fluids were assayed by the real-time Q PCR technique for parasitic *B1* gene. Genotypes at *IL6* -174 G>C as well as *IL1A* -889 C>T and *IL1B* +3954 C>T SNPs were determined using a self-designed nested PCR-RFLP assay. Randomly selected PCR products encompassing the studied *loci* were sequenced. All the genotypes were tested for Hardy-Weinberg equilibrium, and *IL1* genotypes - also for linkage disequilibrium. Relationship between the genotypes or haplotypes at studied polymorphisms and the occurrence of congenital toxoplasmosis was estimated using a logistic regression model.
Results GC heterozygotic status at *IL6* -174 G>C SNP was significantly correlated with development of congenital toxoplasmosis and increased the risk of infection with the parasite (OR 4.24, 95% CI 1.24-14.50 in the codominant model, $P \leq 0.050$). Prevalence rates of genotypes at analyzed *IL1* SNPs were similar between studied patients' groups. The occurrence of C alleles at both *IL6* and *IL1B* SNPs was significantly associated with congenital *T. gondii* infection.
Conclusions Genetic modifications located within *IL6* and *IL1* polymorphic sites might contribute to development of congenital toxoplasmosis.

Poster abstracts

Abstract number: 19

Polymorphisms of genes encoding Toll-like receptors and cytokines and development of congenital cytomegaly

W.I. Wujcicka[1], E. Paradowska[2], M. Studzinska[2], Z.Z. Gaj[1], J.Sz. Wilczynski[1], D.E. Nowakowska[1]
[1]Polish Mother's Memorial Hospital - Research Institute, LODZ, Poland
[2]Institute of Medical Biology, Polish Academy of Sciences, LODZ, Poland

Wujcicka W[1,2], Paradowska E[3], Studzińska M[3], Gaj Z[1,2], Wilczyński J[2], Nowakowska D[2]
Scientific Laboratory of Center of Medical Laboratory Diagnostics, Polish Mother's Memorial Hospital - Research Institute (PMMH-RI), Lodz, Poland
Department of Perinatology and Gynecology, (PMMH-RI), Lodz, Poland
Laboratory of Molecular Virology and Biological Chemistry, Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland
e-mail: wujcickaw@wp.pl

Purpose Determination of distribution of genotypes and alleles located within genes encoding Toll-like receptors (TLRs) and cytokines among congenitally HCMV infected and uninfected fetuses and neonates.
Methods Study included 20 offsprings with HCMV infection and 33 uninfected patients. Anti-HCMV antibodies' levels and IgG avidity were estimated by CLIA and ELFA tests. Quantitation of viral DNA was performed by real-time Q PCR assay for *UL55* gene. Genotype variability at single nucleotide polymorphisms (SNPs) residing within *TLR2*, *TLR4*, *TLR9* as well as *IL1A*, *IL1B*, *IL6*, *IL12B* and *TNFA* genes was assayed by self-designed nested PCR-RFLP techniques. Selected genotypes were confirmed by sequencing. All the genotypes were tested for Hardy-Weinberg equilibrium and *TLR4* as well as *IL1* genotypes were investigated for linkage disequilibrium. Association between genotypes and occurrence of congenital infection was determined using a logistic regression model.
Results *TLR2* 2258 G>A, *TLR4* 1196 C>T and *TLR9* 2248 G>A polymorphisms were significantly associated with occurrence of congenital cytomegaly ($P \leq 0.050$), and GA heterozygotic statuses at *TLR2* and *TLR9* SNPs increased the risk of infection (OR 10.33, and OR 4.00; respectively). The presence of A allele was more frequent among the infected than uninfected offsprings ($P \leq 0.050$). Multiple-SNP analysis showed GCA and CT variants at *TLR4* / *TLR9* and *IL1A* / *IL1B* SNPs, respectively, as correlated with the infection ($P \leq 0.0001$).
Conclusions Changes within *TLR* and *IL1* genes might contribute to development of congenital cytomegaly.

Poster abstracts

Abstract number: 20

Evaluation of the Virocult® liquid transport swab for the detection of Herpes Simplex Virus using the BD Max™ and Smartcycler™ PCR systems

KK Khan, MS Stone, HLJ Jones
Frimley Health NHS, SLOUGH, United Kingdom

Herpesviridiae, the herpes family of viruses that infect humans, consists of 8 separate species, all have double stranded linear DNA genomes enclosed in an icosahedral capsid and lipid envelope.Herpes simplex virus-1, HSV-1 and Herpes simplex virus-2, HSV-2 cause common, self-resolving infections of the skin or mucosa. Classic HSV-1 and HSV-2 clinical findings are described as painful grouped vesicles on an erythematous base, usually with ulcerated and crusted lesions. Both viruses may subsequently reactivate to cause recurrent disease in the face of existing immunity. Although separate species, these viruses cause similar histologic and clinical findings, and speciation relies on laboratory investigation. Generally, HSV-1 has been associated with Orolabial disease, and HSV-2 with genital disease, however, it is possible for HSV-2 to cause Orolabial herpes and HSV-1 to cause genital herpes.The aim of this study was to assess the compatibility of the Virocult® transport swab (Medical Wire and Equipment for the detection of HSV-1 and HSV-2 on both the BD Max™ and Smartcycler™ automated platforms. During this study an updated method of using 200µl of sample on the BD Max™ was used for this study instead of the recommended 500µl volume. This improvement proved to be advantageous when limited volumes of samples were available.
A total of 31 clinical samples and one negative control (molecular biology grade water) were included in this study. The clinical samples were collected for the purpose of detection of the presence of HSV-1 and HSV-2 and comprised of swabs from various sites, including labial swabs, genital ulcer swabs and urethral swabs.Of the 31 Sigma Virocult® samples tested on the BD Max™ the specificity and sensitivity was 100% when compared with the Smartcycler™.

Poster abstracts

Abstract number: 21

Molecular fingerprint of mussel enterobiome is associated with the geographical location

C.C. Orelio[1], R te Witt[2], B. Vis[1], H.E. Dekter[1], M.C. Morsink[1], S Tektas[1]
[1]Hogeschool Leiden, LEIDEN, Nederland
[2]Erasmus Medical Center, ROTTERDAM, Nederland

C.C.Orelio[1], M.L.Seijn[1], R.Nivillac[1], A.Gittenberger[2], W.B. van Leeuwen[1]
[1] Innovative Molecular Diagnostics, University of Applied Sciences Leiden, The Netherlands [2] GiMaRIS, The Netherlands

Traceability of the origin of seafood, including shellfish, is an important issue for the seafood industry and product quality certification. Several studies have shown that the microbiome reflects the geographical location origin. In this study the molecular Automated Ribosomal Intergenic Spacer Analysis (ARISA) PCR method has been applied to determine whether the mussel enterobiome (gut microbiome) can give rise to a location-dependent molecular fingerprint. Therefore, we investigated the mussel enterobiome variation between and within a geographical location.
From two geographical distinct locations ten mussel enterobiomes at various time points were analyzed with the ARISA method. Briefly, DNA extracted from the mussel enterobiome was amplified with primers directed against the 16S and 23S gene region. PCR fragments were subsequently analyzed with capillairy electrophoresis. Bacterial community fingerprints were compared using BioNumerics and PermANOVA statistical analysis software. Moreover, 16S gene Sanger sequence analysis for individual bacterial species was performed to gain additional information on the bacterial population composition.
Our results show that the bacterial composition of the mussel enterobiome results in a molecular fingerprint that allows discrimination between two distinct geographical locations, although also variation between individual from a similar location was observed. Upon transfer of the mussels to a new geographical location, monitoring the enterobiome composition over time revealed that after 4 weeks the newly introduced mussel enterobiome composition resembles the enterobiome composition of the resident mussels. Sequence analysis revealed several common marine, soil and human intestinal bacterial species present in the mussel enterobiome.
It can be concluded that molecular fingerprint analysis of the enterobiome allows discrimination of geographical location of origin of mussels.

Poster abstracts

Abstract number: 22

Comparing the technical performance of two real-time PCR kits for the detection of Trichomonas vaginalis in the laboratory

PW Willems[1], MR Raymaekers[1], BP Peeters[1], KM Magerman[1], LW Waumans[1], BV Vanmassenhove[2], RC Cartuyvels[1]
[1]Jessa hospital, HASSELT, Belgium
[2]Az Damiaan, OOSTENDE, Belgium

Objectives
In the present study, the technical performance of the Trichomonas vaginalis Real-Time® PCR kit of Diagenode (Liège, Belgium) and the Trichomonas vaginalis Real-TM® kit of Sacace Biotechnologies (Como, Italy) is compared.
Methods
DNA extraction was performed using a QIAsymphony® SP extraction robot (Qiagen inc., Hilden, Germany). The extracted DNA was analyzed with both PCR kits using the Rotor-gene® Q analyzer (Qiagen inc., Hilden, Germany). Fifteen *Trichomonas vaginalis* (TV) positive and ten TV negative samples, detected with an in-house validated PCR method in the Az Damiaan hospital, were reanalyzed with both PCR kits in order to evaluate sensitivity and specificity. Specificity was further evaluated by analyzing American Type Culture Collection strains and external quality controls of seven different genital pathogens, other than TV. A serial dilution of commercially available, quantified TV DNA (AMPLIRUN® TRICHOMONAS VAGINALIS DNA CONTROL, Vircell, Granada, Spain) was analyzed with both PCR kits in order to evaluate linearity and to create a calibration curve. To determine the limit of detection (LoD_{95}), this calibration curve was used to retrospectively quantify a TV positive sample. Next, this quantified TV DNA was diluted near the expected limit of detection and each dilution step was analyzed in tenfold, spread over eight days.
Results
The PCR kits of Diagenode and Sacace detected TV DNA in 14 (93%) and 15 (100%) out of 15 TV positive samples, respectively. With neither PCR kit, false positive results were obtained. The efficiency, slope and correlation coefficient obtained with both PCR kits met the criteria suggested by Raymaekers et al. in 2009. The LOD_{95} of the PCR kit of Diagenode and Sacace was 100 copies/mL and 50 copies/mL, respectively.
Conclusion
In this laboratory evaluation, we found no big differences in technical performance of both PCR kits for the detection of *Trichomonas vaginalis*.

Poster abstracts

Abstract number: 23

Comparison of the Performance and Workflow of the Aptima HIV-1 Quant Dx Assay and the COBAS® Ampliprep/COBAS® Taqman HIV-1 Test for Quantitation of HIV-1 RNA.

M Terlouw-Klein, N.C.T. Otten, S.Q. van Veen, D. Kwa
Onze Lieve Vrouwe Gasthuis, AMSTERDAM, Nederland

Background. HIV-1 RNA quantitation using molecular assays is the current gold standard for the management of HIV-infected patients. This study compared the performance of the Hologic Aptima® HIV-1 Quant Dx (Aptima HIV) assay, which uses real-time Transcription Mediated Amplification, and the Roche COBAS® AmpliPrep/COBAS® Taqman HIV-1 version 2 assay (CAP/CTM), which uses real-time PCR. Assay performance was compared in clinical samples (secondary tubes [ST], n=1120 and primary tubes [PT], n=483). Also workflow parameters, such as hand-on time, automation time, turn around time, return visits and time to first results, were compared using clinical samples on both systems.
Results. Quantitative results were highly correlated between Aptima-HIV ST and CAP/CTM ST (n=114; R^2=0.9615; 93% within the 95% CI [-0.3819, 0.7595]), and between Aptima-HIV PT and CAP/CTM ST (n=45; R^2=0.9636; 98% within the 95% CI [-0.3541, 0.7101]). Workflow parameters for CAP/CTM and Aptima HIV (63 clinical samples) were hands-on time of 75 vs. 24 minutes and a total turnaround time of 9h19m vs. 4h22m, respectively. The time to first results was 6h20m vs. 3h05m and automation time 9h19 vs. 4h22. Both assays required one return visit during the run.
Conclusions. The Aptima HIV assay is a good alternative for quantitative measurement of HIV-1 RNA as the assay shows a very good correlation to the CAP/CTM assay with a considerable reduction in hands-on time even more when primary tubes are loaded, a shorter time to results, and continuous sample loading making test results earlier available.

Poster abstracts

Abstract number: 24

Analysis of bacterial DNA load and biomarkers from blood to predict severity of Community-Acquired Pneumonia

AJM Loonen, J Ewalts, R Kusters, P.C Wever, AJC Van den Brule
Jeroen Bosch Hospital, DEN BOSCH, Netherlands

Streptococcus pneumoniae is the major cause of community-acquired pneumonia (CAP) worldwide. It remains difficult to identify CAP patients who are at risk of developing major complications such as septic shock. *S. pneumoniae* DNA load combined with a (set of) biomarker(s) may assist in identifying CAP patients who need special care.
In this pilot study, we analysed *S. pneumoniae* DNA load, C-reactive protein (CRP), procalcitonin (PCT), neutrophil-lymphocyte count ratio (NLCR) and soluble urokinase plasminogen activator receptor (suPAR) in patients of whom a pneumococcal antigen test (BinaxNow) was available.
One hundred patients were included in the study, 45 with a positive and 55 with a negative pneumococcal antigen test. Of the antigen positive patients, 24 had a negative blood culture and 11 had a positive blood culture. Of 10 patients no blood cultures were taken and in 5 of these patients a positive PCR result was obtained. A negative PCR result corresponded in 92% of the cases with a negative blood culture result. In the group with a negative antigen test, 40 patients had a negative blood culture and 39 of these 40 were also negative in the PCR (98%). CRP and PCT values were significantly higher in patients with a positive pneumococcal antigen test ($p < 0.002$). Bacterial DNA load, NLCR and suPAR values were not significantly different between the groups.
In conclusion, in the pneumococcal antigen positive and negative group blood culture and PCR results were similar in 83% and 93%, respectively. Follow up samples are currently being analysed.

Poster abstracts

Abstract number: 25

Development and evaluation of a fluorescent pentaplex assay for microsatellite instability in colorectal cancer

MG Goh, CXQ Chen, CKS Chan, LKH Lim, LO Oon
Singapore General Hospital, SINGAPORE, Singapore

Development and evaluation of a fluorescent pentaplex assay for microsatellite instability in colorectal cancer

Michelle Goh, Eileen XQ Chen, Kian Sing Chan, Tony KH Lim, Lynette LE Oon

Department of Pathology, Singapore General Hospital, Singapore

Introduction:
Lynch Syndrome (LS) is caused by deleterious germline mutations in a set of DNA mismatch repair (MMR) genes. MMR deficiencies are indicated by microsatellite instability (MSI) as microsatellites are most susceptible to replication errors. Existing methods to screen for LS include immunohistochemistry staining (IHC) and PCR-based MSI methods. Here we present the development and evaluation of a single multiplex PCR with fragment analysis modified from methods reported by Buhard *et al*. (2004).
Materials and Methods:
Our in-house assay is a multiplex PCR for the 5 mononucleotide markers (BAT25, BAT26, NR21, NR24 & NR27) and Penta D in a single reaction. The MSI is determined by capillary electrophoresis in ABI 3500 Genetic Analyzer.
In total, 44 colorectal cancer samples were evaluated. There were 6 proficiency test samples, 4 LS samples from a reference laboratory and the remaining 34 samples which all had IHC staining of 4 MMR proteins (MLH1, MSH2, MSH6 & PMS2). In addition, 29 of these samples had MSI testing with a commercial kit by Promega MSI v1.2 or by using the NCI (National Cancer Institute) panel.
Results:
Complete concordance (100%, 44/44) in results was obtained between the in-house method and the reference results. The concordance rate between IHC and the in-house PCR was 94% (32/34) while the concordance rate between the in-house method and the commercial assay (Promega MSI v1.2) was 100% (17/17).
Conclusion:
The use of a single multiplex fluorescent MSI assay reduces the time and costs involved in MSI testing with good reliability and accuracy and thus should facilitate universal screening for microsatellite instability in patients with colorectal cancers.

Poster abstracts

Abstract number: 26

CE-IVD validation of a new molecular diagnostics sample-to-result solution in combination with transplant monitoring RT-qPCR assays

AE Estampes, CB Bittoto, ME Marco, SP Patanè, FG Gorreta, CO Cristina, GS Stefanuto, WM Mahoney
ELITechGroup Molecular Diagnostics, PUTEAUX, France

Objectives: ELITE InGenius™ (ELITechGroup Molecular Diagnostics) is a fully automated sample-to-result solution developed for molecular diagnostics and introduced with a transplant pathogen monitoring menu. The validation study was performed in combination with VZV, HSV1 and BKV ELITE MGB® assays (EMD) with whole blood, plasma and urine samples.
Methods: ELITe InGenius™ automatically performs nucleic acid extraction, amplification and results analysis integrating in a single platform 12 extraction modules and 12 independently controlled thermocyclers. VZV, HSV1 and BKV ELITe MGB® assays are RT-qPCR assays based on MGB technology. The validation plan for each parameter was based on (1) analytical studies with efficiency, linearity, accuracy, repeatability and reproducibility testing to verify the PCR performance; (2) target and internal control sensitivity study; and (3) system performance verification using certified reference material (Qnostics Ltd.). The clinical study included diagnostic sensitivity and specificity was assessed by testing spiked samples (n≈30) and negative blood donor samples (n≈60). The studies were performed in parallel on three ELITe InGeniusT™ instruments.
Results: VZV, HSV1 and BKV ELITe MGB® assays passed the validation criteria of analytical and clinical studies. The analytical sensitivity for each assay was verified at 10 cp/reaction. The clinical sensitivities were: 98%, and 96,7% for VZV and HSV1 respectively with whole blood samples, and 100% for BKV with urine and plasma samples after discrepant resolution. A 100% specificity was obtained for the three assays.
Conclusion: The satisfying results obtained support the CE-IVD marking of ELITe InGenius™ system in combination with VZV, HSV1 and BKV ELITe MGB® assays for the detection and the quantification of DNA extracted and amplified from whole blood, plasma and urine.

Poster abstracts

Abstract number: 27

Molecular epidemiology and drug resistance of Acinetobacter baumannii isolated from ICU in hospitals of southern Poland.

A Chmielarczyk, M Pilarczyk-Zurek, D Romaniszyn, M Pobiega, J Wojkowska-Mach
Jagiellonian University Medical College, KRAKOW, Poland

Objective
Carbapenems resistant Acinetobacter baumannii (AB) is now isolated more frequently, particularly in intensive care settings. The objectives of study were investigate the carbapenemase genes by real-time PCR and determine the molecular epidemiology of the AB strains coming from hospitals from southern Poland.
Material and Methods
The study comprised 125 non-repetitive AB isolates. Among them 99 came from pneumonia, 22 from bloodstream infection and 4 from meningitis. 79.2% strains were from the ICU. Antimicrobial susceptibility testing was performed according to the EUCAST guidelines.. Multiplex real-time PCR was used to screening of the bla_{OXA} genes: bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51} and bla_{OXA-58}. Rep-PCR typing was performed using the DiversiLab *Acinetobacter* kit.
Result
Sixty percent of AB strains were resistant to all studied antimicrobials with the exception of colistin. One isolate was resistant also to colistin. Resistance to carbapenems was: imipenem 80.8% and meropenem 82.4%.
All investigated strains had bla_{OXA-51} gene. 81% of the strains derived from ICU patients had bla_{OXA-24} gene (vs. 53.8% from no-ICU). Among the 101 strains of XDR-AB 80 had $bla_{OXA-24.}$Gene bla_{OXA-23} had 26.4% of all strains. We identified 6 clones and 12 unique strains by rep-PCR. . Two clones were dominated: clone 1 included 24 isolates and clone 2 included 55 isolates. Both of these clones are classified as the international clone II.
Conclusions
The study of drug resistance showed that more than half of strains were resistant to all studied antimicrobials except of colistin. In our study the most commonly was detected $bla_{OXA-24/40-like}$ gene (75%), $bla_{OXA-58-like}$ gene has not occurred at all. Rep-PCR is suitable for genetic comparison of AB isolates, demonstrated good discrimination ability and has automated format. Closely related isolates belonging to IC I-III are predominant in ICUs. Our observations confirmed membership of 63.2% strains to IC II.

Poster abstracts

Abstract number: 28

MolecularDiagnostics.be 2014 external quality control scheme on the molecular detection of Mycoplasma genitalium and Trichomonas vaginalis

PDS Descheemaeker[1], B Vanmassenhove[2], S Pereyre[3], M Raymaekers[4], I Micalessi[5], T Crucitti[6], D Ursi[7], O Pontesilli[8], N Pinon[9], L Coorevits[10], M Reynders[11]
[1]AZ Sint-Jan Brugge-Oostende, BRUGGE, Belgium
[2]AZ Damiaan Oostende, OOSTENDE, Belgium
[3]Hôpital Pellegrin, BORDEAUX, France
[4]Jessa Hospital, HASSELT, Belgium
[5]Imelda Hospital, BONHEIDEN, Belgium
[6]Institute Tropical Medicine, ANTWERP, Belgium
[7]University hospital Antwerp, ANTWERP, Belgium
[8]Cliniques du Sud Luxembourg, ARLON, Belgium
[9]Maasstad hospital, ROTTERDAM, Netherlands
[10]University Hospital Ghent, GHENT, Belgium
[11]AZ St-Jan Brugge-Oostende, BRUGES, Belgium

Mycoplasma genitalium (MG) and *Trichomonas vaginalis* (TV) infections are considered important emerging sexually transmitted infections potentially associated with reproductive tract sequelae, PID, preterm labor, infertility, non-gonococcal urethritis, epididymitis, and prostatitis.
No external quality assessment schemes were available in 2014 for the detection of MG and/or TV. One of the goals of the non-profit organization MolecularDiagnostics.be is to provide quality control samples especially for these parameters where QC samples are not readily available
The current MG/TV ring test was build-up of (i) four diluted MG/TV DNA samples (Vircell Amplirun) enabling to estimate the sensitivity of the assay independently of extraction, (ii) a negative sample and (iii) six MG/TV positive clinical samples enabling to estimate the quality and efficiency of extraction and amplification.
Among the ten participating laboratories 4 different extraction robots were in use. The calculated equivalent of the original sample applied to the PCR reaction varied between 4.6µl to 100 µl. Results did not indicate that this higher input resulted in more sensitive reaction.
The target genes pdhD, MgPa, m219 and TV specific repetitive sequence, G3 hypothetical protein gene, beta tubuline gene were in use for the detection of MG and TV respectively.
All participating laboratories performed equally well in detecting 2,5 copies TV-MG DNA/µl indicating an efficient amplification process. However, considering the simulated clinical samples, where pre-analytical factors and extraction efficiency plays an additional but important role, some laboratories missed a few positive samples which is not correlated to the analytical sensitivity of the assay.

Poster abstracts

Abstract number: 30

Evaluation of Copan FecalSwab for detection of enteric pathogens with Nucleic Acid Amplification Techniques

AG Giambra[1], SC Castriciano[2]
, BRESCIA, Italy
[2]Copan Italia, BRESCIA, Italy

Background: Diarrheal diseases are a leading cause of children and adults mortality. Appropriate specimen collection and transport devices are essential to improve detection of enteric microbes. Copan FecalSwab is an LBM device, consisting of a flocked swab and a tube with 2ml Cary-Blair medium, that can be used for culture with manual and WASP automated plating, for antigen and toxin detection and for nucleic acid amplification assays. In this study was validated the ability of FecalSwab (FS) to stabilize nucleic acids in stools for the detection of Adenovirus, Rotavirus and Norovirus with molecular assays.
Methods: In this validation were used 120 clinical sample of stools, known positive Rotavirus, Adenovirus, Norovirus and negatives. The flocked swab was used to transfer stools in two FS medium tubes. One sample was tested at 0T and after 2 weeks at 4^0C and the other was stored at -80^0C and tested after 6 months. A 200ul aliquot of each FS sample was used for nucleic acid extraction. Five uL of each nucleic acids were tested on the ABI 7500 Real Time PCR System with the R-Biopharm RIDA®GENE Viral Stool panel II and RIDA®GENE Norovirus I & II assays.
Results: Good Adenovirus, Norovirus and negative results correlation and no inhibition were found at 0T, after 2weeks at 4^0C and 6 months at -80^0C in all FS stool samples tested with the RIDA®GENE Viral Stool panel II and Norovirus I & II assays.
Conclusions: The FS kit is compatible with the RIDA®GENE Viral Stool panel II and Norovirus I & II Real-Time PCR assays. The FS can be used for the collection of stool samples for the detection of Adenovirus, Rotavirus and Norovirus nucleic acids

Poster abstracts

Abstract number: 31

MSwab Allows Viral Detection with Direct-Rapid Nucleic Acids Amplification and Culture Assays

S Castriciano[1], A Giambra[1], K Luinstra[2], C Rutherford[2], M Smieja[2]
[1]Copan Italia, BRESCIA, Italy
[2]St Joseph's Healthcare, HAMILTON, ONT, Canada

A.Giambra, S. Castriciano[3], K. Luinstra[1], C. Rutherford[1], and M. Smieja[1.]
[1] St. Joseph's Healthcare and McMaster University, Hamilton ON, Canada.
Copan Italia Spa, Brescia Italy

Background: MSwab is a molecular medium for the collection, and storage of clinical specimens for the detection of viruses with direct-rapid or traditional nucleic acid extraction and amplification assays and culture. Study's objectives were to validate MSwab™ for: 1) Direct-rapid and traditional nucleic acids extraction for the detection of viruses by real-time PCR. 2) Virus isolation by shell-vial culture.
Methods: Nasopharyngeal (N=80) and lesion swab (N=30) were used for this validation. Swabs (N=10) from positive samples, first tested by real time PCR, including Flu A , Flu B, RSV, P1, P2, P3, Adeno, hMPV, HSV1, HSV2, and VZV, were transferred in in a tube of MSwab medium. Each MSwab tube was vortexed and 200 µl of sample were used to inoculate a shell vial culture, another 200 µl were added to a microtube and placed in a dry heating block at 100^0C for 5min, vortexed for 10s and centrifuged at 14,000rpm for 2min. A 200ul aliquot was extracted with the easyMag™ and eluted in 55ul. Five ul of each nucleic acids extracted with both methods were tested with the in-house multiplex real time PCR and compared to the UTM results.
Results: MSwab direct-rapid and EasyMag nucleic acid extraction tested by real-time PCR and shell-vial culture confirmed all FA FB, RSV, P1, P2, P3, ADV, HMPV, HSV1, HSV2 and VZV. No toxicity or contamination was observed in culture.
C**onclusions:** MSwab can be used for direct-rapid nucleic acid extraction for the detection of viruses by real-time PCR and culture. MSwab direct-rapid extraction improves results turnaround time, save costly extraction reagents and supports culture confirmation.

Poster abstracts

Abstract number: 32

Microfluidic-based non-enzymatic glycation enhances the cross-linking of human scleral tissue compared to conventional soaking

JHS Shin[1], CSJ Choi[2], JKH Jin[2]
[1]Kyung Hee University Hospital at Gangdong, SEOUL, South-Korea
[2]Kyung Hee University, SEOUL, South-Korea

Purpose: This study was aim to evaluate nano-structural and chemical change of human scleral collagen by non-enzymatic glycation using atomic force microscopy (AFM), Raman spectroscopy and microfluidics.
Methods: Twenty 8×2 mm scleral strips were divided four groups; the pure sclera tissues (control group, n=5), the scleral tissues ($BSS+DR_7$ group, n=5 and $BSS+DR_{30}$ group, n=5) treated sequentially with the incubation (for 1 hr in BSS and D-ribose) and preservation (for 23 hr in 90% ethanol) during 7 and 30 days in RT, and the scleral tissues ($BSS+DR+\mu F_7$ group, n=5) treated with the incubation (for 1 hr in BSS and D-ribose) and preservation (for 23 hr in 90% ethanol) during 7 days in a microfluidic chip. The $BSS+DR_7$ and $BSS+DR_{30}$ groups were incubated in the mixture of balanced salt solution (BSS) and a 0.2-M ribose in PBS, pH 7.4 containing 0.1% sodium azide while the $BSS+DR+\mu F_7$ group were incubated through supplying the same solutions to two inlet reservoirs of a microfluidic chip.
Results: The sclera tissues incubated with BSS and D-ribose for 7 days in the microfluidic environment ($BSS+DR+\mu F_7$) showed clearly an irregular parallel arrangement of collagen fibrils with tangled fibrils. In glycation groups ($BSS+DR_{30}$, $BSS+DR+\mu F_7$), the Raman shift was observed at 919 cm^{-1}. n-enzymatic glycation led to an increased in the density of both corneal and scleral stromal collagen.
Conclusions: Our method using nonenzymatic glycation in microfluidic environment successfully induced collagen cross-linking. Based on our in vitro results glycation can be used to strengthen connective tissues in several diseases.

Poster abstracts

Abstract number: 33

Simplexa™ C. difficile Direct-A Sample-to-Answer Solution for Labs Combining Real-Time PCR Detection with Simplified Workflow

MM Tabb, A Bologa, Y Parocua, R Martin, S Dempsey, C Cheng
Focus Diagnostics, CYPRESS, United States of America

Objectives *Clostridium difficile* infection (CDI) is a leading cause of hospital-associated gastrointestinal illness. Simplexa™ *C. difficile* Direct is a nucleic acid amplification test for real-time PCR detection of *C. difficile* directly from unformed stool without separate nucleic acid extraction. The assay's sample-to-answer format and simplified workflow allow testing of 1 to 8 samples simultaneously in about an hour. This study evaluated the assay's analytical reactivity, cross reactivity, and limit of detection (LoD). Simplexa *C. difficile* Direct performance was also compared with Cepheid Xpert® *C. difficile* and enriched culture.
Methods LoD study was performed with toxigenic *C. difficile* strains ATCC 43255 & NAP1A. 126 bacteria or virus in stool matrix were tested for cross reactivity. 20 toxigenic *C. difficile* strains were evaluated for analytical reactivity. Potentially interfering substances were tested to determine whether any inhibition was observed. Reproducibility was tested with medium and low positive samples. 100 clinical samples were evaluated with Simplexa *C. difficile* Direct, Xpert *C. difficile* and enriched culture for between-method agreement.
Results Simplexa *C. difficile* Direct LoD was 0.5 CFU/mL for ATCC 43255 and 1.3 CFU/mL for NAP1A. No cross reactivity was observed with the panel of 126 organisms tested. Analytical reactivity testing showed all 20 toxigenic *C. difficile* strains were detected. No interference was observed from any of substances tested. Inter- and intra-assay reproducibility study yielded <5% CV. Positive and negative clinical agreement was 96.7% and 95.7% between Simplexa *C. difficile* Direct and Xpert *C. difficile.* When comparing with enriched culture, positive and negative clinical agreement was 93.1% and 94.4%.
Conclusions
Simplexa *C. difficile* Direct provides an option for simplified *C. difficile* testing on the Integrated Cycler. Simplexa *C. difficile* Direct has convenient workflow and good analytical reactivity compared to Xpert *C. difficile* and enriched culture.

Poster abstracts

Abstract number : 34

Biochemical fingerprints of human papillomavirus infection and cervical dysplasia using cervical fluids: Spectral patterns

YH Kim[1], SJ Choi[2]
[1]The Catholic University of Korea, UIJEONGBU GYEONGGIDO, South-Korea
[2]Department of Biomedical Engineering, College of Medicine, Kyung Hee University, SEOUL, South-Korea

The primary screening tool for invasive cervical cancer, caused by persistent infection with oncogenic human papillomavirus (HPV), is the Papanicolau (Pap) smear. However, this assessment not only cannot discriminate between HPV infection and cervical dysplasia but also results in a low sensitivity. Therefore, we introduce the preliminary finding regarding the label-free, early detection of HPV infection and cervical dysplasia using human cervical fluids. Three experimental groups, such as the HPV-negative group (HPV-, control), HPV-positive group (HPV+), HPV-positive cervical dysplasia group (HPV+CD), were evaluated by a 2-μL drop-coating deposition surface-enhanced Raman scattering (DCD-SERS) method. The proposed screening method yielded the Raman spectra with high reproducibility, noise-independence, and uniformity. It showed similar pattern of Raman spectral intensities and peaks in the ring zone regardless of HPV infection or cervical dysplasia, although there were some insignificantly different patterns in those. The optical detection of HPV infection and cervical dysplasia in cervical fluids could be detected accurately in the central zone. The control group showed a strong intensity of Raman peaks at 877 cm^{-1} (symmetric C-C stretching in lipids) and 963 cm^{-1} (phosphate) compared to reference Raman peak at 1003 cm^{-1} (phenylalanine symmetric ring breath). The HPV+ groups showed a strong intensity of Raman peak at 1448 cm^{-1} (C-H deformation in DNA/RNA, proteins, lipids and carbohydrates), although their Raman patterns showed a similar to those in the ring zone. They showed the lowest difference between the central and ring zones among three groups. The HPV+CD groups showed a distinct spectral difference compared to the control and HPV+ groups. Furthermore, different Raman spectra were obtained according to HPV type. Therefore, all ranges of cervical fluid-induced Raman-spectra could be used to detect the presence of cervical pre-cancer. Raman peak-gated assessment provides a label-free and nondestructive tool for clinical diagnostics of HPV infection and cervical precancerous changes.

Poster abstracts

Abstract number: 35

Whole genome sequences of two endemic multi-drug resistant Pseudomonas aeruginosa isolates

W.B. Kraak
Maasstad Ziekenhuis, ROTTERDAM, Nederland

Whole genome sequences of two endemic multi-drug resistant Pseudomonas aeruginosa isolates
Bart Kraak, Arjan Burggraaf, Wil Goessens, Walter Pirovano, Jacobus M Ossewaarde, Jan Tommassen, Anneke van der Zee
VIM-producing *P. aeruginosa* are well known for their ability to cause outbreaks in hospitals. In the Netherlands, outbreaks due to this multi-resistant organism have been described and reported for the Rotterdam area. Two major AFLP types were observed. The representative isolates Carb01 63 and S04 90 were subjected to whole genome sequencing to enable further investigation into the genetic background of these isolates.
Whole genome sequencing yielded two distinct genomes of respectively 7.5Mbp and 7.1Mbp and a single plasmid in strain S04 90. Both strains contain multiple bacteriophages, are CrispR-Cas deficient and carry *blaVIM-2* on a Class I integron. Differences were observed in the genetic organisation of the *blaVIM-2* gene located on the chromosome of Carb01 63 in comparison to the plasmid located *blaVIM-2* (S04 90). Despite the homology in the organisation of these integrons, small differences in the promoter of *Int1* and the corresponding *aacA* genes were observed. The *aacA29e* gene found in Carb01 63 is different from previously described *aacA* genes. Heterogeneity observed in the integron sequences, indicate different origins for both isolates.
Plasmid encoded *blaVIM-2* was confirmed by obtaining mutants after conjugation experiments. Conjugation experiments showed that antibiotic resistance can be acquired when the plasmid is transferred to a carbepenem susceptible strain PAO-1. Besides whole genome sequencing, growth curves were analysed to investigate the growth of the two WGS strains, PAO-1 and PAO-1 with plasmid. Similar growth rates were found independent of genome size.
Whole genome sequences were submitted to GenBank, accession numbers CP11317 (Carb01 63), CP11369 (S04 90) and CP11370 (plasmid S04 90). Integron structures are filed at INTEGRALL for both strains.

Poster abstracts

Abstract number: 39

THE PERFORMANCE AND EFFECT OF SAMPLE STORAGE TIME ON RESULTS OF A NOVEL C. DIFFICILE TEST SYSTEM EVALUATED IN THREE EUROPEAN LABORATORIES

PM Matero[1], SB Boisset[2], RV Vuento[3], HT Tuokko[4], JK Kauppila[4], LV Venho Liisa[3], MH Huttu Marika[3], SA Ansaranta[4], TM Malila[4], MM Maurin[2], CS Siebert[2], SS Sjöblom[1], JE Elgh[1], JH Hirvonen[3], MM Mäki[1]
[1]Orion Diagnostica Oy, ESPOO, Finland
[2]Grenoble University Hospital Centre, Laboratoire de Bactériologie, GRENOBLE, France
[3]Fimlab Laboratories, TAMPERE, Finland
[4]Nordlab Oulu, OULU, Finland

The performance characteristics of the novel Orion GenRead® C. difficile test (Orion Diagnostica Oy) was studied at three European clinical microbiology laboratories. The accuracy of the test was evaluated by a method comparison against diagnostic tests routinely used in the participating laboratories. The high overall agreement, sensitivity and specificity values (91.9%-100%) of the Orion GenRead C. difficile test against the compared methods are shown in Table 1. In addition, no significant difference in the performance was detected between faecal samples collected either in conventional specimen containers or in transport media (Table 1).
The effect of the turnaround time from sampling to result reporting or a prolonged storage of the faecal samples was also studied. The observed false negative results were assessed not to be caused by longer sample storage time. Thus, it was concluded that storing the samples at +4°C, even up to 26 days, prior to analysis did not affect the Orion GenRead C. difficile test performance (Table 2).
As a summary, 1160 faecal samples from patients suspected of having Clostridium difficile infection were analysed with the Orion GenRead C. difficile test which proved to be a robust, sensitive and specific method for detecting toxigenic C. difficile in faeces. The test can be performed on faecal samples collected into either conventional specimen containers or in transport media.

Table 1. Method comparison results

Methods		Orion GenRead C. difficile		Total no. of samples	Positive agreement	Negative agreement	Overall agreement
		Positive	Negative				
illumigene® *C. difficile*	Positive	79	7	397	91,9 %	98,1 %	96,7 %
	Negative	6	305				
		Positive	Negative	Total			
IMDx *C. difficile*	Positive	23	2	170	92,0 %	100 %	98,8 %
	Negative	0	145				
		Positive	Negative	Total			
IMDx *C. difficile**	Positive	68	6	437	91,9 %	99,7 %	98,4 %
	Negative	1	362				
		Positive	Negative	Total			
C. DIFF QUIK CHEK + Xpert® *C. difficile*	Positive	14	1	156	93,3 %	97,2 %	96,8 %
	Negative	4	137				

* Samples collected into FecalSwabs

Picture 1: Table 1. Method comparison result Table 1. Method comparison results

Table 2. Effect of storage time on the Orion GenRead C. difficile test performance

Time of sample storage from sampling to analysis	No. of analysed samples			No. of false negative samples		
	Nordlab Oulu	Fimlab	Grenoble CHU	Nordlab Oulu	Fimlab	Grenoble CHU
The same day	63	30	56	-	-	1
1 day	221	151	34	6	2	-
2 days	77	170	14	-	2	-
3 days	21	113	21	1	2	-
4 days	14	72	3	-	2	-
5 days	-	21	22	-	-	-
6 days	-	6	3	-	-	-
7 days	-	1	2	-	-	-
8 days	-	-	1	-	-	-
12 days	1	-	-	-	-	-
17–26 days	-	44	-	-	-	-
Total No. of samples	**397**	**607**	**156**	**7**	**8**	**1**

Picture 2: Table 2. Effect of sample storage time on the Orion GenRead C. difficile test performanc Table 2. Effect of sample storage time on the Orion GenRead C. difficile test performance

Poster abstracts

Abstract number: 41

Reverse Transcription Strand Invasion Based Amplification (RT-SIBA) Method for Rapid Detection of Human Rhinoviruses

KE Eboigbodin[1], V Kainulainen[2], T Ojalehto[1], S Elf[1], R Korpela[2], P Susi[3], M Mäki[1]
[1]Orion Diagnostica Oy, ESPOO, Finland
[2]Medical Faculty, Pharmacology, University of Helsinki, HELSINKI, Finland
[3]Department of Virology, University of Turku, TURKU, Finland

Human rhinoviruses (HRVs) are the most common cause of respiratory illness, and major cause of morbidity in infants, elderly and immunocompromised individuals. They are associated with severe infections requiring hospitalization, especially with acute otitis media, wheezing and asthma. Diagnosis of HRVs also has importance in preventing antibiotics misuse. Real-time reverse transcription polymerase chain reaction (RT-qPCR) has been developed for the diagnosis of HRVs, offering improved sensitivity over the time-consuming virus culture method. However, RT-qPCR requires thermal cyclers and skilled personnel, which consequently limit its use in field or point-of-care applications.

We previously described a novel isothermal nucleic acid amplification method called Strand Invasion Based Amplification (SIBA®) with high analytical sensitivity and specificity (1). The method relies on the recombinase-dependent insertion of a single-stranded, invasion oligonucleotide (IO) for the dissociation of the target duplex. This event allows target specific primers to bind and extend the target via the action of a DNA polymerase. The method was previously found to be useful for the rapid detection of DNA from pathogens. In this study, we developed a reverse transcription SIBA (RT-SIBA) for the rapid detection of viral RNA targets. RT-SIBA assay was designed to detect the conserved sequences within the 5' untranslated region of HRV genome. The method further includes a reverse transcriptase enzyme that allows a one-step reverse transcription of RNA to cDNA and simultaneous amplification and real-time detection of cDNA with SIBA. The reaction was performed at low and constant temperature (41^{o}C) eliminating the need for the repeated cycles of heating and cooling steps.

The RT-SIBA was able to detect low copies of HRV RNA within 30 minutes. RT-SIBA was found to be a rapid, specific and sensitive method for the detection of viral RNA and has great potential as a powerful diagnostic tool in centralised and decentralised test settings.

Poster abstracts

Abstract number: 42

The newly developed Cepheid Xpert® HIV-1 VL compared to the established Abbott RealTime HIV-1 viral load measurement

R Ehret, A Moritz, M Schuetze, MJ Obermeier
MIB, BERLIN, Germany

Background:
We compared the newly developed GeneXpert® HIV-1 VL-assay in the known cartridge format with the random access capability to the broadly used Abbott m2000 RealTi*m*e HIV-1 viral load-assay. Special focus was put on reproducibility of results in the low viral load range and linearity in HIV-1 B and non-B-subtypes.
Methods:
Fresh (n=100) and diluted (n=225) patient samples were tested. Daily clinical routine samples were tested on the same day in parallel. Three high viral load samples from different HIV-1 subtypes (B, CRF01_AE, CRF02_AG) were diluted to target-concentrations from 100.000 cop./ml to 15 cop./ml. Each dilution step was tested in 5 replicates. To better assess reproducibility in the low ranges, dilutions with 250 cop./ml, 125 cop./ml, 63 cop./ml were tested in 10 replicates.
Results:
79 of the fresh samples were below the detection limit of 40 cop./ml in the Xpert®, 82 with RealTi*m*e respectively. While only 11 of those samples showed a detection signal, 22 did with Xpert® leading to an overall concordance of 77%.Intra- and inter-assay variation was very low and comparable up to superior to RealTi*m*e with intra-assay %CV ranging from 2.5% with viral loads of 2.4 log cps/ml to 7.8% with 1.8 log cps/ml.Linearity on the diluted samples could be show by using a simple linear regression model with a slope of 0.99, intercept of 0.07 and a coefficient of determination (R^2) of 0.99.
Conclusions:
A high correlation between the Abbott m2000 RealTi*m*e and the Xpert® HIV-1 VL could be shown. The higher detection rate below quantification limit might hint to a slightly higher sensitivity of the Xpert®. The assay showed excellent linearity and reproducibility.The random access capability and the rapid time to result of only 90 minutes make the GeneXpert® platform and the Xpert® HIV-1 VL a valuable tool in clinical routine, especially for urgent samples.

Poster abstracts

Abstract number: 43

Human papillomavirus and Chlamydia trachomatis testing on same specimen

EY Padalko, LS Seaux, EP Padalko
Ghent University Hospital, GHENT, Belgium

Human papillomavirus and Chlamydia trachomatis testing on same specimen

Seaux[1], E. Padalko[1,2]
[1]Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium
[2]School of Life Sciences, Hasselt University, Diepenbeek, Belgium

Introduction. As different specimens are necessary for human papillomavirus (HPV) and *Chlamydia trachomatis* (CT) molecular testing (cervical smear and cervical/vaginal swabs respectively), the objective of our study was to explore the possibility of performing PCRs for both agents on same sample. Methods. Based on e-query samples for CT and HPV testing 14 or 21 days before and after routine HPV and CT PCRs respectively were searched retrieving a total of 26 patients with sufficient volume for additional testing and at least one positive result for CT and/or HPV. Abbott® CT RealTime PCR and Abbott® High Risk HPV RealTime PCR were performed on swabs and smears. Results. HPV on swabs: for 12 patients, swabs missed 2 from 8 high risk HPV other than HPV16 or HPV18 (other hrHPV); missed HPV16 in one sample and other hrHPV in another from 2 samples positive for HPV16 and other hrHPV and detected additional other hrHPV in one of the 2 patients positive for HPV18. CT on smears: all CT results on swabs and smears of 14 patients were similar except of one CT-positive patient who was CT-negative on smear. This patient had twice a CT PCR result beyond cutoff representing weak signal, smear was taken 14 days after the CT sampling while patient was treated, possibly explaining the negative result. Conclusions. This pilot study shows promising results, especially regarding CT testing on cervical smears. More superficial nature of swabs can be a possible explanation of the inferior results of HPV testing of these samples. Further studies are needed to explore possibility of same specimen collection for HPV and CT testing.

Poster abstracts

Abstract number: 44

The relationship between Ct-value in Clostridium difficile positive feces samples and fecal consistency

M.J.C.A. van Trijp, P. van Wunnik, L.C. Smeets
Reinier de Graaf Gasthuis, DELFT, Nederland

Introduction

Broad spectrum antibiotic therapy can cause overgrowth of *Clostridium difficile* (Cd) disturbing the normal fecal flora. However, presence of Cd in feces can be difficult to interpret as Cd can be present as a part of the normal commensal flora in 2-5% of the population. Quantification of Cd-load might help to distinguish Cd associated diarrhea from asymptomatic carriage. Therefore, we collected clinical data from fecal samples that had tested positive for Cd DNA by real-time PCR in our laboratory and tried to relate fecal consistency to the Cycle threshold (Ct).

Methods

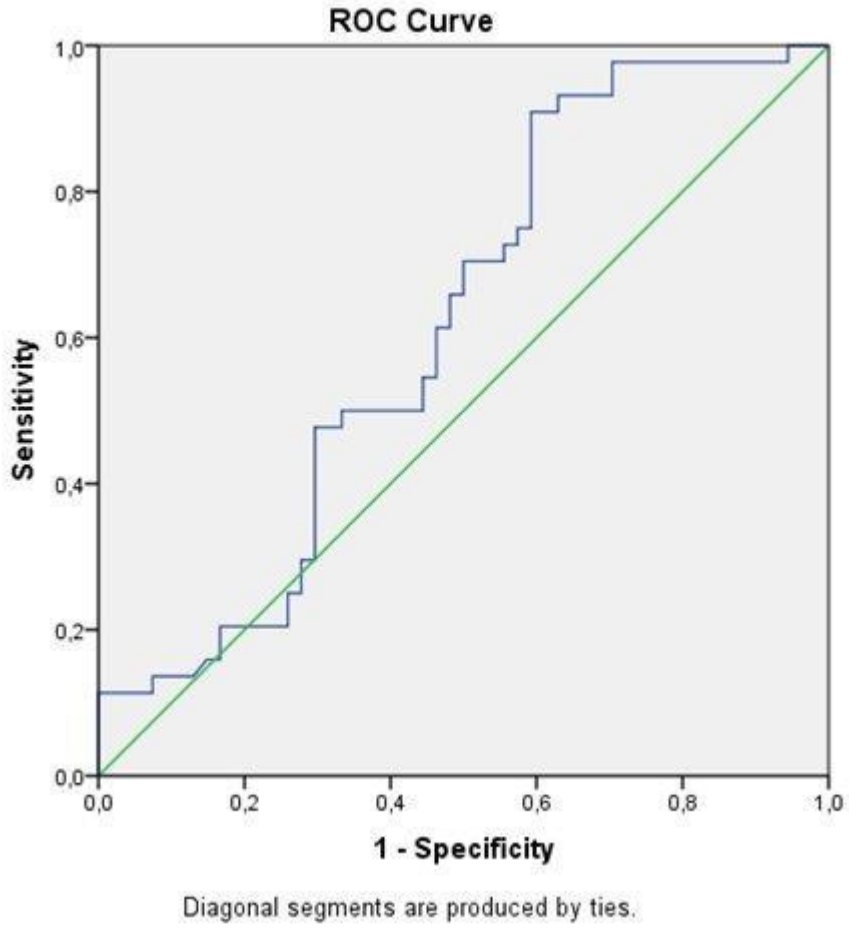
We performed a retrospective observational study. Clinical data were collected from fecal samples which had tested positive for Cd DNA. For detection of Cd DNA the *tcdA* and *tcdB* gene were amplified. The study period ranged from august 2013 through September 2014. The aim was to investigate whether a relationship could be detected between Ct-value level and fecal consistency as observed by the laboratory technician.

Results

18 samples were excluded because of missing data on consistency. 6 samples were excluded due to a vague description of consistency. Of the remaining samples, those with normal consistency had a median Ct-value of 30 (n=51; range 24-41), whereas diarrheal samples had a median Ct-value of 28 (n=44; range 23-39). Performing ROC-curve analysis using SPSS software did not result in defining a suitable cut-off value. (Area under the curve 0.618)

Conclusion

In our study population no relation was found between Ct-value and consistency in *Clostridium*-DNA positive fecal samples. No cut-off value for a clinical significant Ct-value could be determined.



Poster abstracts

Abstract number: 45

The relationship between detected Ct-value in Salmonella enterica positive feces samples and fecal consistency

M.J.C.A. van Trijp, P. van Wunnik, L.C. Smeets
Reinier de Graaf Gasthuis, DELFT, Nederland

Introduction

Presence of *Salmonella enterica* (Se) in feces can be difficult to interpret as it can indicate infection or carriage. The amount of published data on the topic of relation between fecal bacterial load (colony number) and clinical disease is limited. Therefore we collected clinical data from fecal samples that tested positive for Se DNA in our laboratory by real-time PCR and tried to relate fecal consistency to the Cycle threshold (Ct).

Methods

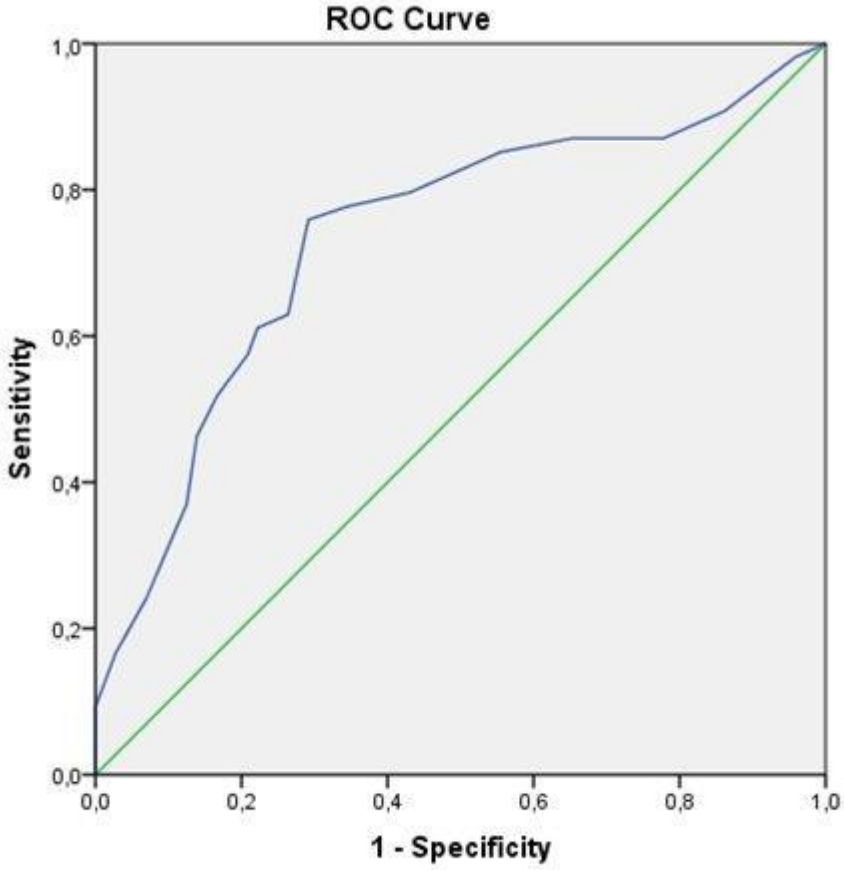
We performed a retrospective observational study. Clinical data were collected on feces samples that tested positive for Se DNA in a real-time PCR on the *invA*-gene. The study period ranged from August 2013 through February 2015. The aim was to investigate whether a relationship could be detected between Ct-value level and fecal consistency as described by the laboratory technician.

Results

7 samples were excluded because of missing data on consistency. Another 7 samples were excluded due to a vague description of consistency. Feces samples with normal consistency had a median Ct-value of 34 (n=71; range 23-39, whereas diarrheal samples had a median Ct-value of 31 (n=46; range 19-38). Performing ROC-curve analysis using SPSS software resulted in an area under the curve of 0.736. At a Ct-value cut-off of 31,5 the sensitivity was 76% with a specificity of 70%.

Conclusion

In our test system, a Ct-value below 31,5 might indicate presence of Se infection. A Ct value of 31,5 or above most likely indicates carriage without diarrhea. However the specificity is low. Therefore we conclude that Ct-value level is not a robust marker to distinguish between *Salmonella* carriage or infection.



Diagonal segments are produced by ties.

Poster abstracts

Abstract number: 49

THE PERFORMANCE AND EFFECT OF SAMPLE STORAGE TIME ON RESULTS OF A NOVEL C. DIFFICILE TEST SYSTEM EVALUATED IN THREE EUROPEAN LABORATORIES

PM Matero[1], SB Boisset Sandrine[2], RV Vuento Risto[3], HT Tuokko[4], JH Kauppila[4], LV Venho[3], MH Huttu[3], SA Ansaranta[4], TM Malila[4], MM Maurin[2], CS Siebert[2], SS Sjöblom[1], JE Elgh[1], JH Hirvonen[3], MM Mäki[1]
[1]Orion Diagnostica, ESPOO, Finland
[2]Grenoble University Hospital Centre, Laboratoire de Bactériologie, GRENOBLE, France
[3]Fimlab Laboratories, TAMPERE, Finland
[4]Nordlab Oulu, OULU, Finland

The performance characteristics of the novel Orion GenRead® C. difficile test (Orion Diagnostica Oy) was studied at three European clinical microbiology laboratories. The accuracy of the test was evaluated by a method comparison against diagnostic tests routinely used in the participating laboratories. The high overall agreement, sensitivity and specificity values (91.9%-100%) of the Orion GenRead C. difficile test against the compared methods are shown in Table 1. In addition, no significant difference in the performance was detected between faecal samples collected either in conventional specimen containers or in transport media (Table 1).
The effect of the turnaround time from sampling to result reporting or a prolonged storage of the faecal samples was also studied. The observed false negative results were assessed not to be caused by longer sample storage time. Thus, it was concluded that storing the samples at +4°C, even up to 26 days, prior to analysis did not affect the Orion GenRead C. difficile test performance (Table 2).
As a summary, 1160 faecal samples from patients suspected of having Clostridium difficile infection were analysed with the Orion GenRead C. difficile test which proved to be a robust, sensitive and specific method for detecting toxigenic C. difficile in faeces. The test can be performed on faecal samples collected into either conventional specimen containers or in transport media.

Table 1. Method comparison results

Methods		illumigene *C. difficile*		IMDx *C. difficile*		IMDx *C. difficile**		C. DIFF QUIK CHEK + Xpert® *C. difficile*	
		positive	negative	positive	negative	positive	negative	positive	negative
Orion GenRead C. difficile	positive	79	6	23	0	68	1	14	4
	negative	7	305	2	145	6	362	1	137
Total no. of samples		397		170		437		156	
Sensitivity / Positive agreement		91.9%		92.0%		91.9%		93.3%	
Specificity / Negative agreement		981%		100 %		99.7%		97.2%	
Overall agreement		**96.7%**		**98.8%**		**98.4%**		**96.8%**	

* Samples collected with FecalSwabs

Picture 1: Table 1. Method comparison result Table 1. Method comparison results

Table 2. Effect of storage time on the Orion GenRead C. difficile test performance

Time of sample storage from sampling to analysis	No. of analysed samples			No. of false negative samples		
	Nordlab Oulu	Fimlab	Grenoble CHU	Nordlab Oulu	Fimlab	Grenoble CHU
The same day	63	30	56	-	-	1
1 day	221	151	34	6	2	-
2 days	77	170	14	-	2	-
3 days	21	113	21	1	2	-
4 days	14	72	3	-	2	-
5 days	-	21	22	-	-	-
6 days	-	6	3	-	-	-
7 days	-	1	2	-	-	-
8 days	-	-	1	-	-	-
12 days	1	-	-	-	-	-
17–26 days	-	44	-	-	-	-
Total No. of samples	**397**	**607**	**156**	**7**	**8**	**1**

Picture 2: Table 2. Effect of sample storage time on the Orion GenRead C. difficile test performanc Table 2. Effect of sample storage time on the Orion GenRead C. difficile test performance

Poster abstracts

Abstract number: 51

HepatiC, the Viral Hepatitis C Patients Management Application and Database.
RB Boulmé
ABL S.A., LUXEMBOURG, Luxembourg

HepatiC, the Viral Hepatitis C Patients Management Application and Database.
R Boulmé[1], M Barralon[1], D Gonzalez[2], C Sayada1
[1] ABL SA, Luxembourg, Luxembourg.
[2] ABL TherapyEdge Spain SL, Barcelona, Spain.

Background
New antiviral treatments are increasingly being evaluated and approved for the management of chronic viral hepatitis C. The related costs and side effects require efficient and reliable data management software systems to collect data and enable clinicians to stratify patients for personalized treatment and monitoring. We developed HepatiC, an Hepatitis C software and database aiming to improve patient management.

Methods
HepatiC has been developed with a multi-sites approach and following high levels of standardization and confidentiality. Over the time clinical information including multiple pre-treatment cycles, liver biopsy, non-invasive fibrosis analysis, comorbidities and lab information are collected through HepatiC. Visits, side effects, new treatments and outcomes are also stored. Data collected are then processed in VisibleChek, an application used to perform data reporting.

Results
HepatiC was announced during the XXXVIII annual congress of the Spanish Association of Liver Disease (AEEH). The knowledge bases are regularly updated with the newest treatments and the software is also regularly updated with new features. In June 2015, HepatiC gathered data from several thousands of Hepatitis C infected patients from more than 10 countries. All the collected data have been imported in VisibleChek for high-level analysis for global epidemiological and surveillance initiatives, including to improve the personalized treatment selection and the cost-effectiveness of HCV-related treatments.

Conclusions
HepatiC is a personalized Hepatitis C disease management software and database validated by experts, with regularly updated knowledge bases and features Used to centralize and share information between medical experts, the combination of HepatiC and VisibleChek can be used to optimize research programs, optimal follow-up of antiviral hepatitis C treatment and improve patients management.

Poster abstracts

Abstract number: 52

EFFICACY AND SAFETY OF PROTEASE INHIBITOR BASED TRIPLE THERAPY IN LATIN AMERICAN HCV GT1 INFECTED PATIENTS (LALREAN COHORT)

RB Boulmé[1], CS Sayada[1], MB Barralon[1], MS Silva[2]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]Hepatology and Liver Transplant Unit. Hospital Universitario Austral, BUENOS AIRES, Argentina

EFFICACY AND SAFETY OF PROTEASE INHIBITOR BASED TRIPLE THERAPY IN LATIN AMERICAN HCV GT1 INFECTED PATIENTS (LALREAN COHORT).

Ronan Boulmé[1], C. Sayada[1], M. Barralon[1], Marcelo Silva MD[2], on behalf of the Latin American Liver Research, Education and Awareness Network (LALREAN).
ABL SA, Luxembourg, Luxembourg
Hepatology and Liver Transplant Unit. Hospital Universitario Austral. Presidente Derqui, Buenos Aires; Argentina.

Background and aims:
Efficacy and safety of peginterferon alfa (P) plus ribavirin (R) combined with boceprevir (BOC) or telaprevir (TVR) for the treatment of hepatitis C genotype 1 in clinical practice seems to be comparable with results of randomized-controlled trials (RCTs). The aim of the study was to evaluate the efficacy and safety of this treatment for Latin-America patients.
Methods:
Medical records of patients treated with triple therapy in Hepatology Units from LALREAN partners were uploaded in HepatiC™ database system for its analysis. Patients <18y, with HIV or HBV coinfection, and those treated in RCTs were excluded.
Results:
We included 141 men (66%), mean age 52.6y, mean HCV RNA 3,898,064 IU/ml, Gt1 24 (11.2%), Gt1a 61 (28,6%), Gt1b 127 (59,6%) and 129 (61%) had F3-F4. 119p were naïve (56%). 100p (47%) were treated with TVR and in this group 68% were cirrhotics and 58% were naïve. In TVR group, 10p are still on treatment. 90p completed treatment with 11 early discontinuation. 80% of patients achieved end of treatment response (EOTR), and in those completing follow up 42% achieved a sustained virological response (SVR) while 23% relapsed and 35% were non responders. Regarding AE, in TVR group 8-36% developed anemia, 15-36% neutropenia, and 13-40% thrombocytopenia. Same indicators have been collected for BOC group.
Conclusions:
Results show that efficacy and safety of triple therapy in a cohort with a high percentage of cirrhotic patients of Latin-America, are similar to those reported in RCTs.

Poster abstracts

Abstract number: 53

Software dependent differences in HIV-1 drug resistance determination.

RB Boulmé[1], DG Gonzalez[2], CS Sayada[1], AS Sung[3], LM Mazur[3]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]ABL TherapyEdge, BARCELONA, Spain
[3]ACL Central Laboratory, ROSEMONT, United States of America

Software dependent differences in HIV-1 drug resistance determination.

Ronan Boulme[1], Dimitri Gonzalez[1,] Chalom Sayada[1], Ah-Young Sung2, Boyka Baltadjieva[2], Michael Michalov[2], Lech Mazur2
[1] ABL SA, Luxembourg; [2] ACL Central Laboratory Rosemont, IL USA..

Introduction Software for HIV-1 genotypic drug resistance testing is routinely used to generate clinical drug resistance interpretations. In this study we compare the differences found in the results obtained with distinct software.

Methods HIV sequencing data of forty five (45) clinical samples belonging to treatment-experienced patients were analysed using ViroSeq (VS) Genotyping Software v3.0.0.32. All (VS) results were compared to the FDA-registered DPM product and to the RUO ViroScore-HIV® system from Advanced Biological Laboratories which include several knowledge databases i.e. Stanford HIVdb v7.0.1 (SD) or the virtual-phenotypic-based algorithm from Geno2Pheno v3.3 (G2P).

Results Overall, G2P was the algorithm showing fewer interpretations classified as “Resistant”(8.9%, compared to 9.4% with SD and 9.2% with VS) and VS was the one showing the highest percentage of “Susceptible”interpretations (86.1%, compared to 75.3% with SD and 78.3% with G2P).

For 41 of the samples we retrieved resistance interpretations for 19 drugs with all three algorithms, allowing us to compare 779 drug resistance results between algorithms. In 34.1% of the samples, VS reported different resistance interpretations for at least one drug when compared to SD, with a 1-level lower resistance value (from Resistant [R] to Intermediate [I] or from I to Susceptible [S]). When considering only the interpretations where SD was in agreement with G2P (714), VS reported 1-level lower resistance values for at least one drug in 12.2% of the samples.

Etravirine, Rilpivirine and Saquinavir jointly account for 53.8% of the differences comparing VS to SD.

Conclusions Laboratories performing DR testing should be aware of alternative interpretive systems which could be used to supplement their existing DR reports.

Poster abstracts

Abstract number: 54

Implementation of the BD MAX™ system for detection of Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis using E-swab™

EVL Vanlaere, SCL Claeys, LVA Van Acker, CV Verfaillie, JVA Van Acker
AZ Sint Lucas, GENT, Belgium

Sexual transmitted infections (STI) are increasing worldwide and are most commonly caused by pathogens such as *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* (TV). A rapid and reliable detection is needed to start directed therapy at the earliest and to prevent further transmission.
In our lab, presence of CT and NG was diagnosed with the ProbeTec**TM** ET CT/NG (BD) molecular assay and TV with direct microscopy. Both methods are laborious and sensitivity is suboptimal, especially for microscopy. A rising number of samples (>3000 samples/year) forced us to a new high-throughput approach. A qualitative multiplex assay, BD MAX™ CT/GC/TV, was developed on BD MAX™ (BD), a platform combining extraction and amplification, processing up to 24 samples/run. Moreover, only 200 µl of sample input is needed. In our study, 462 routinely collected genital samples (E-swab™), 26 urines and 4 extragenital samples were tested using this assay in order to assess the performance characteristics in comparison to our routine approach. Prevalence of CT, NG and TV with BD MAX™ was 3% (16/492), 0.8% (4/492) and 2.2% (11/492) respectively. In addition, one CT and 5 TV were detected using BD MAX™, while our routine approach was negative. The assay was not hampered by the use of E-swab™. The results were available within 3 hours with a minimum of hands-on-time and no particular molecular skills, allowing to perform routine analyses on daily basis.
Based on the increased sensitivity and a high level of automation, the BD MAX™ instrument was implemented for routine STI diagnostics in our lab.

Poster abstracts

Abstract number: 56

Validation of the Cepheid Xpert MRSA Gen3 cassette.

S.J Mulder, P Kuyper-Claessen, T A Schuurs, R van Rhee-Luderer
Izore, centre infectious diseases Friesland, LEEUWARDEN, Netherlands

Background:
Currently, Izore performs rapid detection of MRSA using the Xpert MRSA test (Cepheid). A major drawback of this test is that it doesn't detect all relevant SCCmec types and MecC. Recently, Cepheid launched the Xpert MRSA Gen3 test which detects more SCCmec types and mecA/mecC. In this study the Xpert MRSA test was compared with the Xpert MRSA Gen3 test.
Methods:
Ten fold MRSA dilutions were tested in both the Xpert MRSA and the Xpert MRSA Gen3 cassette.
A selection of Xpert MRSA negative MRSA strains was analysed with the Xpert MRSA Gen3 cassette.
Three MRSA culture negative and nine MRSA culture positive clinical samples were tested with the Xpert MRSA test and the Xpert MRSA Gen3 test.
Results:
Dilution series showed that the Xpert MRSA Gen3 cassette is at least as sensitive as the Xpert MRSA cassette.
All MRSA strains (n=8) which were SCC negative in the Xpert MRSA test showed a positive result in the Xpert MRSA Gen3 test. Two mecC MRSA strains tested mec positive in the Xpert MRSA Gen3 cassette.
When testing clinical samples two MRSA negative materials were negative in both tests and one tested false positive in the Xpert MRSA test. Out of nine MRSA positive samples, four materials were positive in both tests, two were only positive in the Xpert MRSA Gen3 test and three were only positive in the Xpert MRSA test. Three samples had to be reanalyzed after an error of a Xpert MRSA Gen3 cassette.
Conclusions:
Despite satisfactory results on strains, further investigation is needed on clinical samples and the errors before replacing the Xpert MRSA test by the Xpert MRSA Gen3 test.

Poster abstracts

Abstract number: 59

Lyophilized real-time PCR kits from Fast-track Diagnostics: faster and even easier

AH Hagen
ESCH-SUR-ALZETTE, Luxembourg

Annegret Hagen[1], Madeleine Heckmann[1], Miriam Steimer[1], William F. Carman[1]
[1]Fast-track diagnostics, Luxembourg

Background
Purpose of the study was to transfer liquid Fast-track diagnostics real-time multiplex kits into dry ready-to-use formulations. A range of multiplex primer and probe mixes (PPmixes) detecting viruses and bacteria have been lyophilized with Fast-track mastermix in ready to go formulation. Such dry formulations are cheaper to transport and have longer stability.
Methods
FTD Bacterial gastroenteritis, FTD ACE and three multiplexes of FTD Respiratory pathogens 21 were lyophilized with different PPmix and different enzyme concentrations. They were tested with both clinical samples and plasmid dilutions. Lyophilized formulations have been compared with current wet version of Fast-track diagnostics multiplexes. 310 clinical specimens were extracted on NucliSENS® EasyMAG® (bioMérieux), cycling was performed on Applied Biosystems®7500 (Thermo Fisher Scientific).
Results compared to liquid version
Conclusion
The easy to use application of lyophilized Fast-track diagnostics real-time multiplexes is a time saving and more flexible alternative compared to existing liquid multiplexes. By addition of extracted material to lyophilized PCR mixture people save time and are more flexible by testing one or up to 62 patients at the time without discarding reagent.

Poster abstracts

Abstract number: 60

Proficiency testing programmes as a tool to assess and improve diagnostic methods for infectious diseases.

C Di Lorenzo[1], PS Wallace[1], HGM Niesters[2], AM Van Loon[1]
[1]QCMD, GLASGOW, United Kingdom
[2]University Medical Centre Groningen, GRONINGEN, Netherlands

The generation of reliable and reproducible diagnostic test results is of paramount importance in clinical settings as they may allow for infections to be halted before clinical symptoms are apparent. Additionally changes in pathogen load in the patient may indicate the need to modify treatment strategies to prevent further disease progression.
The development of International Standards in some areas such as for the blood borne viruses HCV, HBV, HIV and B19 has helped considerably in improving test reproducibility within the laboratory, which in turn helps facilitate comparison of results across laboratories. However, the lack of suitable reference material or International Standards for some pathogens coupled with a wide range of in-house developed assays utilising different extraction and amplification methods, makes comparison of results across laboratories rather difficult.
In the absence of International Standards, Proficiency testing (PT) can provide a valuable tool to assess and improve diagnostic methods for infectious diseases. The design of the PT challenge allows comparison across multiple laboratories using numerous molecular technologies.
Results on QCMD PT panels for a range of human pathogens showed a trend towards an improvement in performance. The majority of assays used by laboratories showed a high degree of sensitivity. The percentage of false-positive results has been decreasing, although it remained an issue in specific areas, i.e. detection of fungal pathogens. An improvement in the precision of quantitative assays was also observed in recent years.
Commercial assays are becoming the favoured method of choice, particularly as regulation around diagnostic laboratories is introduced. However, in-house tests still remains an important part of the diagnostic arsenal, particularly where commercial assays are not readily available. A move towards the use of real time assays was also evident whilst the use of non-PCR methods was uncommon.

Poster abstracts

Abstract number: 61

Validation and implementation of Next Generation Sequencing in pathology diagnostics

JH Radersma, W.W.J. De Leng, J.W.J. Hinrichs, C.M.A. De Voijs, W.T.M. Van Blokland, M.J. Koudijs, N.J.M. Besselink, F.A.S. Smouter, I. Nijman, S.M. Willems, R.A. De Weger
UMC Utrecht, UTRECHT, Netherlands

Introduction Personalized medicine and the accompanying targeted therapies ask for a change in approach regarding the molecular analysis of tissue samples. Instead of single gene analysis, gene panels can be analyzed to accurately determine whether patients are eligible for a specific therapy. Next Generation Sequencing (NGS) allows for the simultaneous analysis of mutations in multiple genes and is therefore a potential platform to use in diagnostics.
Aim Validation and implementation of NGS in pathology diagnostics.
Methods NGS was tested using the AmpliSeq Cancer Hotspot Panel (50 genes) and the IonTorrent PGM platform. 150 samples were tested retrospectively and validation was continued with prospective testing of 100 samples. Validation needed to prove whether the run time, reliability, cost effectiveness, data analysis, translation of data into clinical reports and logistics were satisfactory.
Results An entire NGS workflow from FFPE tissue selection till data reporting (bioinformatics) could be completed within a week (two runs/week). Several discordant results were identified, which could all except one be explained by incomplete exon coverage in the gene panel. Reliable results were obtained when the tumor percentage >10%, DNA quantity >20 ng, >500 reads, mutation identified with a coverage >5% in both forward and reverse reads.
Conclusion The IonTorrent PGM is a reliable platform that can be used in pathology diagnostics. With incomplete exon coverage in the NGS run, additional conventional mutation analyses could be necessary. NGS can be reliably used in pathology diagnostics if technical work, bioinformatics and logistics are performed in a well-coordinated and collaborative way.

Poster abstracts

Abstract number: 62

Copan eNAT Medium Stabilize Nucleic Acids for the Detection of Viruses with the xTAG Respiratory Virus Panel

ALICE SQUASSINA[1], arnalda Giambra[1], santina Castriciano[1], K.L. Luinstra[2], S.M. Smieja[2]
[1]COPAN ITALIA, BRESCIA, Italy
[2]St. Joseph's Healthcare and McMaster University, HAMILTON, ONTARIO, Canada

eNAT is a molecular medium that preserves nucleic acids and inactivates viral viability. It can be used for collection and transportation of clinical samples for detection of infectious pathogens with nucleic acid amplification assay. eNAT is available in tubes with 1 or 2 ml or with flocked swabs for specific sample collection sites.
Objectives: To validate nucleic acids stabilization in eNAT for the detection of viruses with the Luminex xTAG Respiratory Virus Panel (RVP).
Methods: Two viral pools, prepared with clinical strains of viral isolates, titrated in UTM and inNAT, were used to inoculate both media. The first pool included Flu A (H3N2), Flu B, and RSV B, the second included Adeno, HMPV and Para 2. 100µl of each pool was added to 1ml UTM, and to 1ml eNAT tubes and tested at T0, after 2W and 4W at 4^0C and RT, and 8W at-20^0C and -80^0C. At each time, 200µl of both UTM and eNAT samples was used to extract nucleic acid on the EasyMAG (Biomerieux). 5µl of extracted samples was added to RVP-1 master mix and tested on RVP-1 assay.
Results: All viruses (Flu A (H3N2), Flu B, and RSV B, Adeno, HMPV and Para 2) were detected in eNAT and UTM at all times and conditions. Viral nucleic acids were stable in eNAT medium at all testing times.
Conclusions: Copan eNAT can be used for collection and long term storage of clinical specimens for the detection of respiratory viruses with the RVP assay. eNAT medium is optimal for specimen's collection from patients with high risk infectious pathogens.

Poster abstracts

Abstract number: 63

Technical validation of three commercial real-time PCR kits for the diagnosis of neuroborreliosis in cerebrospinal fluid on three different real-time PCR platforms.

M Raymaekers[1], T Carolus[2], R Cartuyvels[1], L Braeken[2], P.-J. D'Huys[2], L Maes[2]
[1]Jessa Hospital, HASSELT, Belgium
[2]Res. group Lab4U, Dep. of Industrial Science and Technology, UC Leuven-Limburg, DIEPENBEEK, Belgium

Lyme neuroborreliosis is caused by the spirochete *Borrelia burgdorferi sensu lato* complex (*Bb sl*). Diagnosis mainly relies on interpretation of clinical signs and serology. The goal of this study is to evaluate the technical performance of three commercially available assays [*Borrelia burgdorferi* PCR kit (Geneproof), *Borrelia burgdorferi sensu lato* Real-TM kit (Sacace) and the O-Dia-Borburg real-time PCR kit (Diagenode)] using three different real-time PCR platforms [Rotorgene Q (Qiagen), CFX96 (Bio-Rad) and LightCycler (Roche)] in order to select a method suitable for clinical validation.
DNA was extracted using Qiasymphony SP (Qiagen). Performance characteristics such as specificity, inclusivity, limit of detection ($LOD_{95\%}$), linearity and reproducibility were evaluated using EQC panels (Instand), ATCC strains and commercially available DNA (Vircell). Linearity, reproducibility and $LOD_{95\%}$ were determined for *Borrelia afzelii, garinii* and *sensu stricto*. Aliquots for $LOD_{95\%}$ measurements were preserved at 4°C and -20°C to mimic transport and storage conditions.
No cross-reactivity was found for genetically related organisms or for pathogens which may be present in CSF. All species of the *Bb sl* complex were detected with Geneproof and Sacace. Diagenode failed to detect *B. lusitaniae*. $LOD_{95\%}$ measurements indicate a better sensitivity than described in the kit insert. All kits showed a larger linear range on Rotorgene Q than on CFX96 and Lightcycler. A good reproducibility was obtained for all assays. Preliminary results seem to indicate a better overall performance of Geneproof on Rotorgene Q.

Poster abstracts

Abstract number: 64

Novel End-to-end Sequencing Solutions for Sanger and Next Generation Sequencing (NGS) of HIV and Viral Hepatitis C (HCV).

RB Boulmé[1], DG Gonzalez[2], MB Barralon[1], CS Sayada[1]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]ABL TherapyEdge, BARCELONA, Spain

Introduction: The management of patients infected with HIV or Viral Hepatitis C (HCV) relies on an accurate viral genomic profiling. Molecular assays combining reagents and powerful data analysis software are on demand by clinical diagnostics labs. We present the DeepChek HIV and HCV Assays.
Discussion: Targeting key HIV and HCV regions and suited to any Sanger or NGS platform, the DeepChek Assays embed all the reagents required for a robust viral sequences amplification. It also include an analysis software producing clinically-interpretable reports. HIV-1 Reverse transcriptase, Protease and Integrase amplicons obtained from the HIV assay as well as NS5B amplicons from the HCV assay have been generated from a panel of hundreds of well-characterised frozen clinical plasma samples from the Caribbean region, Brazil and from Europe. On an average, the HIV Assay succeeded to amplify all the available subtypes (B, C, F, A1, D, 02_AG…) with viral loads ranging from 2000 to 1.000.000 copies/mL. Even better sensitivity (above 50 copies) is being obtained through optimized versions of the protocol. The HCV Assay showed equivalent performances in term of specificity (subtypes 1a, 1b, 3, 4…) and sensibility. Amplicons were sequenced using two methodologies (Sanger sequencing with Big Dye kits on one hand and NGS with Illumina Nextera XT and Miseq on the other hand). Sequences were analysed with ViroScore® and DeepChek® technology respectively where clinical genotyping reports (combining genotypes, mutations, and drug resistance assessment) were automatically generated. All results were coherent with previous samples characterization.
Conclusion: We developed new robust end-to-end solutions combining reagents and software systems directly compatible with diagnostics interpretation of HIV and HCV infection.

Poster abstracts

Abstract number: 65

Change in viral shedding associated with hypertonic saline nasal irrigation and gargling: ELVIS, a pilot randomised controlled trial

S Ramalingam[1], C Graham[2], J Dove[3], L Morrice[4], A Sheikh[4]
, EDINBURGH, United Kingdom
[2]Wellcome Trust Clinical Research Facility, EDINBURGH, United Kingdom
[3]Department of Laboratory Medicine, NHS Lothian, EDINBURGH, United Kingdom
[4]The University of Edinburgh, EDINBURGH, United Kingdom

Background and Methods: Indirect evidence suggests that hypertonic saline nasal irrigation and gargling (HSNIG) may improve virological and clinical outcomes in viral upper respiratory tract infections (URTI). We conducted a pilot randomised controlled trial (RCT) titled Edinburgh and Lothians Viral Intervention Study (ELVIS) comparing HSNIG vs. usual care to assess the feasibility of undertaking a definitive RCT. We randomised 68 otherwise healthy adults within 48 hours of URTI to HSNIG at the highest comfortable concentration of hypertonic saline (1.5%-3.0%) with Cornish Sea Salt (Intervention) or usual care (control). Symptom diaries were completed until well for two consecutive days or a maximum of 14 days. A self-taken flocked midturbinate swab (Copan) was collected in eNAT medium (Copan) at baseline and first thing on days 1-4 to measure viral shedding. Day 0 swabs were tested for 15 viruses by in-house PCR. Where virus was detected, all 5 samples were tested in parallel to determine change in viral shedding
Results and discussion: Clinical data (to be presented at ESCV, 2015), showed a significant reduction in duration of illness (p=0.01), over-the-counter medication use (p=0.04) and illness in members of household after index case (p=0.04) in the intervention arm. Here we report participant feedback, correlation of viral shedding with symptoms and HSNIG.
82.4% and 100% of participants reported swabs were very-moderately easy to collect and return respectively, though controls thought swabs were more difficult to collect (7% vs. 24%, p=0.045). A virus was identified in 72.7% of participants (rhinovirus 58.3%**;** coronaviruses 31.3%). 8.3% had multiple viruses. Viral shedding correlated well with changes in symptom score across viruses. HSNIG was associated with reduction in viral shedding, explaining both early recovery and reduction in transmission within household in the intervention arm.

Poster abstracts

Abstract number: 68

New Bioinformatics System Suited for the Management of Sanger and Next Generation Sequencing (NGS) Human Cytomegalovirus (HCMV) Data.

RB Boulmé[1], MB Barralon[1], DG Gonzalez[2], CS Sayada[1]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]ABL TherapyEdge, BARCELONA, Spain

Introduction
Even if HCMV infection is usually benign, it can be life-threatening for immunocompromised patients. As antiviral resistance usually occurs in transplant recipients, sensitive and accurate genotyping platforms, suited for both Sanger and NGS technologies are required.
Methods
We developed the DeepChek-CMV platform and analysed 26 clinical samples. Sanger sequences covering regions UL54 and UL97 were submitted for genotyping and drug resistance determination and compared with results obtained through an in-house methodology. Mutations detection was carried-out through the built-in variant calling process of the platform. Drug resistance determination for Cidofovir, Foscarnet, Ganciclovir and Maribavir was determined though several embedded guidelines. Relevant information were summarized in clinical reports suited for clinical interpretation by pathologists.
Results
Among the 26 clinical samples included in the study, 23 UL54 and 23 UL97 genotypes and interpretations were obtained. The most prevalent UL97 mutations conferring drug resistance, DRM were 592G, 594V, 460V, 520Q, 460I, 595F, 595S, 595W and 594P found at 26%, 22%, 13%, 9%, 9%, 9%, 4%, 4%, 4% of the UL97 genotypes respectively whereas only the 413E DRM was observed in 3 UL54 genotypes (13%). Drug resistance interpretations were found in 13%, 0%, 77% and 0% of the possible interpretations for Cidofovir, Foscarnet, Ganciclovir and Maribavir. All the results were consistent with and in range with the diagnostics previously determined using a well-validated homebrew methodology.
Conclusions
A comprehensive and accurate panel of analyses including genotypes characterization for HCMV UL54 and UL97 regions, drug resistance interpretations were carried-out though an easy-to use format using the DeepChek-CMV pipeline helping clinical diagnostics laboratories in the management of Sanger HCMV data and their transition to NGS.

Poster abstracts

Abstract number: 69

Molecular Detection of Mycobacterium tuberculosis: 5 Years of External Quality Assessment

E McCulloch, C Di Lorenzo, P Wallace
GLASGOW, United Kingdom

Mycobacterium tuberculosis is a fastidious and slow growing bacterium which can be difficult to culture in the laboratory. Due to the public health implications of delayed diagnosis where the organism can spread easily amongst vulnerable groups, accurate and specific diagnostics ensure infections are detected early and patients can be managed appropriately.
QCMD distribute *Mycobacterium tuberculosis* proficiency panels annually. Laboratory results for these programmes (2010-2014) are presented below. The panels contained different concentrations and of *M. tuberculosis* complex covering in different matrices cover the main clinical specimen types sputum and cerebrospinal fluid (CSF). The panels are distributed to laboratories worldwide. The results are collected through a dedicated online reporting system, before being analysed by QCMD to determine laboratory performance and determine trends in analysis over time.
The number of laboratories participating in this programme increased from 172 in 2010 to 235 in 2014. Real-time PCR is the predominant technology used increasing from 69% to 91%. There has however been a shift from in-house developed to commercial assays with 65% of results generated from commercial assays in 2010 and 78% in 2014. Overall performance has increased with 72.3% of participants correctly identifying all core samples correctly in 2010 and 91.6% in 2014. A review of comparable results over the years shows an improved rate of detection particularly in complex respiratory specimens where a sample containing 2000 copies/ml detection improved from 86% to 99% in 2014. A low false positivity rate of between is consistent with other EQA programmes provided by QCMD.
While culture remains the ‘gold standard’ for *M. tuberculosis* diagnosis the advantage of timely return of results provided by molecular methods is clear and improved diagnosis can only improve patient management.

Poster abstracts

Abstract number: 70

Molecular identification of species belonging to Aspergillus section Nigri isolated in clinical samples (FUNGAE Study Group)

F Galán[1], C Castro[2], D Miralles[1], MJ Linares[3], C Pazos[4], ML Serrano[5], W Sanchez-Yebra[6], E Martin-Mazuelos[2], M Rodriguez-Iglesias[1]
[1]Puerta del Mar Univ Hosp, CADIZ, Spain
[2]Virgen de Valme Univ Hosp, SEVILLE, Spain
[3]Reina Sofia Univ Hosp, CORDOBA, Spain
[4]San Pedro de Alcantara Hosp, CACERES, Spain
[5]Virgen de las Nieves Univ Hosp, GRANADA, Spain
[6]Torrecardenas Hosp, ALMERIA, Spain

The identification of cryptic species of *Aspergillus* is not possible by examining the phenotypic characteristics and mass spectrometry (MALDI-TOF) is not still able to distinguish closely related species. The aim of the study was to identify, using molecular techniques, cryptic species *Aspergillus* (section *Nigri*) in a prospective multicenter study of invasive fungal infections performed in Southern Spain.
Thirty-five isolates from 10 hospitals in southern Spain were identified morphologically and by mass spectrometry (MALDI-TOF, Bruker Daltonics) as *Aspergillus niger.* Genomic analysis was performed by PCR and partial sequencing of the beta-tubulin gene with a double primer pairs (βtb2a / βtb2b and βtub1 / βtub4) previously described. The consensus sequences were compared to the reference sequences available in the Genbank database using the BLAST tool.
Genomic sequencing analysis has identified five clades: *A. tubingensis* (22 strains), *A. niger* (10 strains), and one strain of each of the following specie: *A. aculeatus, A. awamorii,* and *A. carbonarius.* Of all 35 clinical isolates, 31 strains (88.5%) were identified by mass spectrometry as *A. niger* and 4 strains (11.4%) were not identified. Only 25.7% of the isolates showed matching results between the genomic and proteomic analysis, while the rest belonged to cryptic species not included in the database library of filamentous fungi 1.0 Bruker Daltonics.
Although, in the section *Nigri, A. niger* is the species most frequently associated with invasive fungal infections and aspergillomas, in Spain there is a clear predominance of *A. tubingensis* (62.85%) against *A. niger* (28,57%) already described in other studies and confirmed by us. At present it is not possible to identify with accuracy the cryptic species of *Aspergillus* section *Nigri* by proteomic analysis with data libraries available. It is therefore necessary to use sequencing techniques to identify species most prevalent in our geographic area as *A. tubingensis.*

Poster abstracts

Abstract number: 71

Recovery of Genomic DNA and isolation of 16S Ribosomal DNA from Sigma Transwab® system with viability acceptance in accordance with CLSI M40-A2 standard

N Gizzie, E Adukwu
University of the West of England, BRISTOL, United Kingdom

Sigma-Transwab® PF is a liquid medium format transport swab designed for use on automated processing platforms. The medium within is liquid Amies and the swab is flock, which allows for greater absorption and release of bacterial cells. In 2014 the revised standard CLSI M40-A2 included new provisions for the evaluation of liquid medium transport swab transport systems with novel bud types such as foam and flock. In this study the ability of the Sigma Transwab PF® to recover genomic DNA was assessed; The quality, yield and purity of the DNA was determined using the Nanodrop 1000, PCR was used to demonstrate amplification of 16S ribosomal DNA primers. In addition Sigma Transwab® (foam tip) and Sigma Transwab® PF were evaluated for viability and recovery of all recommended 10 bacteria strains according to the CLSI M40-A2 swab elution and roll plate method.

Genomic DNA was recovered from the liquid media with high yield and quality after the 24hr holding period at RT for all 10 bacteria strains. Analysis via PCR shows successful amplification of ribosomal DNA for all bacterial genera, with no indication of interference or inhibition between the swab/liquid media and the bacteria. Sigma Transwab® and Sigma Transwab® PF met CLSI acceptance criteria for all aerobic, anaerobic and fastidious isolates stored at both temperatures for both elution and roll plate methods.

Both molecular and conventional based processing platforms are becoming more widely used within diagnostic procedures and the Sigma Transwab® system (foam and flock) has demonstrated its ability to be a flexible swab transport system for use on all such systems.

Poster abstracts

Abstract number: 72

Molecular detection of bla TEM and bla SHV genes among clinical isolates of Escherichia coli from Kashan, Iran

A Khorshidi, M Rohani, M Akbari, M Motallebi, H Akbari, M Khorshidi
Kashan University of Medical Sciences, KASHAN, Iran

Molecular detection of *bla TEM* **and** *bla SHV* **genes among clinical isolates of** *Escherichia coli* **from Kashan, Iran**

Khorshidi Ahmad, Rohani Mehdi, Akbari Motahare and Motallebi Mitra*
Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, I.R. Iran.

Introduction : *Escherichia* spp. are opportunistic pathogens that cause nosocomial infections. Because of their acquisition of multi drug resistant plasmids, these organisms are resistant to a number of antibiotics, including extended spectrum cephalosporins and aminoglycosides.

Material and Method :The aim of this study is to detect extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from Shahid Beheshti Hospital in Kashan. This descriptive study was done on clinical specimens isolated from Shahid Beheshti Hospital in Kashan.

Result: Identification of the isolated bacteria was done by standard biochemical testes. Determination of antimicrobial susceptibility was done by disk diffusion method. The ESBL production was investigated on isolates by double disk synergy technique. ESBL producers were confirmed by MIC method. PCR amplification of *ESBL* genes, TEM-1 and SHV-1 was carried out. 150 specimens were *E. coli* and 70 were ESBL (46%). Of the total of 70 specimens isolates, 9 out of 40 (13%) included TEM-1 and SHV-1. 5 specimens (7%) were SHV-1 and 44 specimens (63%) were TEM-1.

Conclusion : Given the high levels of resistance, accurate antibiogram tests before prescribing antibiotics and avoiding indiscriminate use of antibiotics are essential.

Key words: Extended Spectrum Beta-lactamase (ESBL), *Escherichia coli*, nosocomial infections

Poster abstracts

Abstract number: 73

USE OF GS-JUNIOR AND DEEPCHEK TO STUDY ANTIRETROVIRAL RESISTANCE IN HIV PATIENTS

RB Boulmé[1], JC Fernandez Caballero[2], DG Gonzalez[3], CS Sayada[1], FG Garcia[2]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]Complejo Hospitalario Universitario Granada-San Cecilio-PTS, GRANADA, Spain
[3]ABL TherapyEdge, BARCELONA, Spain

BACKGRUND AND AIM
Next Generation Sequencing (NGS) is becoming a fundamental tool for clinical virology labs involved in routine genotyping of HIV patients, as it confers the capacity to rapidly obtain clinically-relevant information. We present the workflow combining 454 GS-Junior with DeepChek-HIV v1.4 in patients diagnosed with HIV.
PATIENTS AND METHODS
Reverse transcriptase, protease and/or integrase were subjected to UDPS using the GS-Junior 454 Roche-kit. Sequences were initially processed with the AVA 454 software. Alignments were further uploaded into the DeepChek-HIV v1.4 (ABL SA) system. For interpretation of resistance the Stanford algorithm (HIVdb version 7.0.1); mutations with a score > = 5 were also recorded.
RESULTS
Pol regions (of 185 HIV positive patients were analyzed (101 samples from naive patients and 84 patients treatment-experienced). Subtype distribution was: B (78%), 02_AG (10%), A1 (4%), F1 (3%), C and D (2%) and G (1%). Using VisibleChek for analysis, we were able to describe the detection of any mutation using a 1% cutoff in 164/185 patients, with a total of 386 mutations with Stanford score >=5. The most prevalent primary mutation for RT was K103N (n=16 patients) and for PR I84V (n=3). Using UDS-1% data, 32/185 patients (17.3%) showed any resistance to NNRTIs, 26/185 to NRTIs (14%) and 10/185 to PIs (5.4%). Using a UDS-20% (Sanger approach) decreased resistance to all classes NNRTI [21/185 patients (11.3%); NRTIs 9/185 (4.8%) and PIs 3/185 (1.6%).
CONCLUSIONS
The implementation of 454 GS Junior and DeepChek-HIV allow easy, intuitive, reliable, and fast (results obtained in 3 days) NGS HIV-1 data analysis and offers to labs involved in HIV routine diagnostics. The DeepChek-HIV application has a direct connection with the data mining VisibleChek software; providing the ability to visualize key information through graphs and to correlate genomic information with clinical data.

Poster abstracts

Abstract number: 74

Infectious Disease Monitoring via a Paperless, Secure, Integrated, Standardised and Current Knowledge Based Application

RB Boulmé[1], FH Havenith[2], MB Barralon[1], CS Sayada[1]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]ABL TherapyEdge, JOHANNESBURG, South Africa

Background
Centralising screening, diagnostic and monitoring data is essential to the ongoing management of infectious diseases. Described is a dedicated electronic platform, TherapyEdge-VisibleChek (TE-VC), CE marked and HIPAA compliant.
Methods
It has been designed to suit private and state practices, non-government organisations, employee programs and academic institutions during paediatric and adult patient consultations, information management and research respectively. Data are exported to VisibleChek for elaborate data mining, visualisation and reporting.
Results
Various mechanisms have been developed to ensure this secure platform meets all data protection requirements. Knowledge bases are current and standardised with real-time updates. Data interoperability occurs with laboratory, pharmacy, billing and short message service (SMS) systems using HL7, web services and other industry standards. Molecular and routine laboratory results match to patient files via unique identifiers saving time, providing real-time access and averting manual entry errors. Sanger and next generation sequencing outcomes from ViroScore and DeepChek respectively populate relevant patient files. During electronic prescription issuing from the patient file, information obtained from abnormal laboratory and genotyping results, conditions and / or drug to drug interactions of already present medication, alert. Integrated TEEval provides flexible package insert type of information for when drug initiation or changes are being considered.
SMSes alert patients to forthcoming and missed visits increasing adherence.
Conclusion
TherapyEdge contributes to common and individual disease management via the ongoing consolidation of and instant access to pertinent data. Used to centralise and share information between medical experts, this combination of TherapyEdge and VisibleChek optimises research programs, follow-up of drug treatment (including cost reduction) and improves patient management.

Poster abstracts

Abstract number: 75

Robust Viral Hepatitis C Subtyping through DeepChek HCV NS5B Assay.

RB Boulmé[1], SM Mohamed[2], HK Khiri[2], DG Gonzalez[3], CS Sayada[1], PH Halfon[2]
[1]ABL S.A., LUXEMBOURG, Luxembourg
[2]Alphabio, MARSEILLE, France
[3]ABL TherapyEdge, BARCELONA, Spain

Robust Viral Hepatitis C Subtyping through DeepChek HCV NS5B Assay.

Ronan Boulme[1], Sofiane Mohamed[2], Hacène Khiri[2], Dimitri Gonzalez[1], Chalom Sayada[1], Philippe Halfon[2]
[1] ABL SA, Luxembourg; [2] Alphabio, Marseille, France.

Introduction: Even if direct-acting antivirals (DAAs) used to treat the Viral Hepatitis C (HCV) infection are very potent, a correct and sensitive stratification of patients, based on infecting viral strains profiling is required to optimize patient management. We present the DeepChek HCV NS5B Assay.

Discussion: Developed through a collaboration between the laboratory Alphabio (France) and Advanced Biological Laboratories (ABL, Luxembourg), the DeepChek HCV NS5B Assay is a diagnostics product combining all reagents suited for the amplification of the NS5B region, the related Sanger sequencing primers and the data analysis software. Suited to any kind of Sanger or Next Generation Sequencing (NGS) platform, the assay has been tested and even used for routine diagnostics purposes for 17 (1998-2015) years in France. On an average, around 5280 clinical samples have been analysed and has been able to sequence a heterogeneous panel of HCV strains comprising subtypes (1-6). The test is quite sensitive as well since viral loads ranging from 1250 to 150 000 000 IU/ml were successfully amplified. The data analysis component is included in the Assay and carried-out through the DeepChek-HCV Software Application which summarizes in a clinical diagnostics report format, key information including genotype(s), subtype(s), co-infections assessment with quantification of related quasi-species.

Conclusion: DeepChek HCV NS5B Assay is a routine diagnostics product, in the process of being CE IVD certified, which can efficiently contribute to a sensitive profiling of HCV infection leading to personalized medicine.

Poster abstracts

Abstract number: 76

A modular software solution (under development) to expedite and mitigate workflow issues in regulatory environments

LSK Kelly, JG Gordon
Illumina, SAN DIEGO, United States of America

One of the challenges of working in regulatory environments is the need to revalidate legacy workflows when incorporating new information or tools. Even in unregulated environments, updating and integrating workflows can require a considerable expenditure of time and labor. To mitigate these issues, Illumina is developing an analysis framework software as part of the Universal Oncology Test for the MiSeqDx™ System. By incorporating plug-in modules that seamlessly integrate with the framework"yet remain self-contained and separately versioned from other modules"the software will expedite and ensure scalable operations. Installing new modules to the framework will incur no need for code changes to the legacy workflows, a key consideration for development and validation in regulated environments. The updated MiSeqDx analysis framework includes user authentication and sample sheet setup, reducing the number of disparate application interactions and bringing them together in a clean, clinical user interface accessible via web browser.
The Universal Oncology Test System software workflow module will allow an investigator to identify variants of interest via three methods: an eligibility list of specific coordinate nucleotide changes, configurable rules based on variant type and region, and an amplification gene list with specified amplification type. This flexible software solution can be used to produce, for example, clinical trial reports that require masking of non-investigational variants while not precluding the opportunity to informatically unfilter new information. A report definition can be applied per sample, and updated post-sequencing for a reanalyzed report. The *in vitro* diagnostic report, also in development, aims to support the pathologist end user by separate categorization of pathogenic variants based on companion diagnostics.
The MiSeqDx analysis software platform combined with the Universal Oncology Test System workflow module can help expedite immediate clinical trial uptake and detection of variants for interpretation and future patient care.

Poster abstracts

Abstract number: 78

A Next-Generation Sequencing Method for Identifying Somatic Variants from FFPE Solid Tumor Samples

ILD Deras, TL Le, AY Young, GC Caves, KC Chang, AD Daulo, CGK Glidewell-Kenney, CLH Hsieh, AJ Jager, CL Lin, XL Luo, JM McLean, SP Pond, JLS Silhavy, EU Upsall, HYC Chuang, LK Kelly, CZ Zhao, MF Friedenberg
Illumina, SAN DIEGO, United States of America

The application of genomics in cancer has led to an improved understanding of the disease. Driver genes have been discovered and their pathways have become the focus of small-molecule drug development, many of which target specific mutations. As more variants are identified to be of clinical significance, the single-analyte model of companion diagnostics for approved therapies can be ineffective. An additional, well-known challenge is the degradation and damage caused to DNA in formalin-fixed, paraffin-embedded (FFPE) samples. We developed a novel method for simultaneous detection of multiple somatic variants from FFPE specimens. This method leverages a multiplexed PCR approach with amplicons designed to a 15-gene panel that can detect somatic variants in solid tumors. In our experiments, this multiplexed PCR assay has been demonstrated to be highly sensitive, yielding results from as little as 20 ng of DNA extracted from FFPE tissue, even when the DNA is highly damaged and degraded. Somatic variants can be detected down to 5% allele fraction. This method, incorporating next-generation sequencing and newly designed software, will be deployed as part of the Illumina Universal Oncology Test System.*
*For research use only. Not for use in diagnostic procedures.

Poster abstracts

Abstract number: 84

Validation and clinical performance of an in-house JAK2V617F mutation detection assay on the Rotor Gene Q

B Vanmassenhove
Az Damiaan, Oostende, Belgium

Background
The presence of JAK2V617F mutation is part of the reference WHO criteria for the diagnosis of polycythemia vera, essential thrombocythemia and primary myelofibrosis in BCR-ABL1 negative patients.

Methods
The mutation is detected by a TaqMan© based real-time PCR with a wild-type blocking LNA (Locked Nucleic Acid) probe, increasing the specificity and sensitivity. DNA quantity and possible inhibition were checked by a second control-qPCR.
Primers and probes were chosen from the literature (Denys et al., J of Molecular Diagnostics, 2010).

DNA from 117 patients (EDTA blood) and 4 external quality controls was extracted using the MagNA Pure platform (Roche, DNA I High Performance protocol). The sensitivity panel of NIBSC and DNA-standards (HorizonDx) were also included in the validation process.
5 µl DNA was added to 20 µl mastermix. The mastermix used was LC480 Probes Master (Roche). The final concentration of the primers and hydrolysis probes was respectively
0.3 µM and 0.1 µM. The LNA-probe had a final concentration of 1 µM.

The assay was checked for accuracy, reproducibility, linearity, clinical sensitivity and specificity following the Belgian guidelines (Raymaekers et al., Acta Clinica Belgica, 2011).

Results
Patients and external quality controls were confirmed in a reference laboratory and were all concordant.
The variation coefficient for a high and low allelic burden sample was respectively 2.99% and 1.46%.

50 routine patient samples, clinically expected negative for JAK2V617F were analysed to determine the clinical specificity, one was weak positive and was later confirmed with 0.2%. allelic burden.

The clinical sensitivity was 1% mutant allelic burden which is in line with the British guidelines (Bench et al., British J of Haematology, 2013).

The mutation and control PCR had an efficiency of 99%.

Conclusion
The cost-effective and rapid assay met all our analytical and clinical validation criteria and is therefore implemented in daily routine.

Poster abstracts

Abstract number: 93

POINT-OF-IMPACT TESTING IN THE EMERGENCY DEPARTMENT: RAPID DIAGNOSTICS FOR RESPIRATORY VIRAL INFECTIONS

Poelman[1], van der Meer[1], Van Leer-Buter[1], Riezebos-Brilman[1], HGM Niesters[2]
[1]University Medical Center Groningen, Groningen, Nederland
[2]University Medical Centre Groningen, GRONINGEN, Netherlands

Background Molecular diagnostics for infectious diseases is increasingly important in a clinical setting for disease management and infection control. We aimed to assess the value of Point-of-Impact (POI) testing for respiratory viral infections at the emergency department by implementing an adequate diagnostic policy.

Methods The Emergency Department (ED), the Department of Medical Microbiology (DMM) and several clinical wards were actively involved. The BioFire respiratory panel (bioMerieux) was implemented and we extended our service from 08 to 22hr, 7 days a week during the respiratory season 2014/2015. We measured both (clinical) benefits and costs. To represent the cost-effectiveness of POI testing in a hospital broad setting, we introduced the €h-concept (comparable with kWh).

Results Between December 11th 2014 and April 5th 2015, 641 tests were requested by the ED. 492 contained the full set of information and showed a mean turnaround time of 2 hours and 2 minutes at the DMM. This has led to a mean total time to result of 3 hours and 14 minutes, from patient registration at the ED to result. 330 (67%) of these patients were admitted to a clinical ward in our hospital and in 93% of these cases, the results were available before admission. This has led to a huge improvement of in-hospital patient flow, an improved (preliminary) diagnosis, a more adequate therapy and we prevented 181 unnecessary isolations. Furthermore, cohorting of patients improved bed management. Therefore, beside the positive test results, this policy also increases the value of samples that have been tested negative.
Despite the slight increase of direct costs at the DMM due to the extended services, there are substantial (clinical) benefits which are estimated to be €150.000.

Conclusions We successfully implemented rapid diagnostics for respiratory viral infections which is cost-effective.

Keywords Diagnostic stewardship, point-of-impact, cost-effectiveness, hospital-broad policy