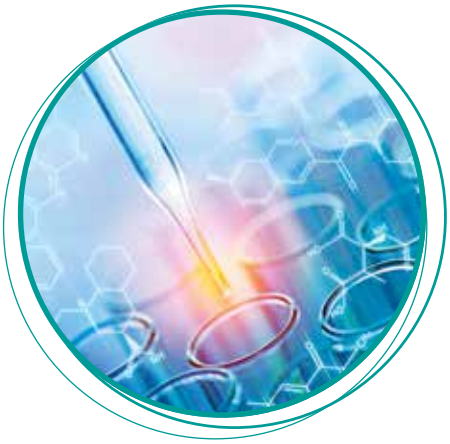
Global Engage

THE RESEARCH & TECHNOLOGY SERIES:

qPCR & DIGITAL PCR / LIQUID BIOPSIES / FLOW CYTOMETRY

LONDON, UK

6-7 December 2021



#randtseries

www.global-engage.com

Global Engage is pleased to announce the **8th Annual qPCR & Digital PCR Congress** as part of the Research & Technology Series, a three-conference event examining the latest in biomarker discovery, diagnostic tools and cellular analysis.

qPCR and dPCR are essential techniques used for quantification of nucleic acid molecules in molecular biology and diagnostics. qPCR is a powerful tool that enables precise and quantitative data reflecting the biology of the tested experimental parameters, while the precision of dPCR allows its application for the detection of rare point mutations and mutations induced by gene editing in a background of wild-type sequences. However, accuracy, reproducibility, assay optimisation, multiplexing, standardisation and translating methods into applications are amongst the challenges that need to be taken into consideration to be able to move forward into clinical settings successfully.

This congress will address these challenges through case studies and interactive sessions on qPCR and dPCR across diverse areas such as oncology, infectious diseases, vaccines, clinical applications, microbiology, and other novel applications. Take the opportunity to be updated with information on current projects and applications of these technologies and serve as an opportunity to further strengthen business partnerships with those in your field.

Research & Technical Considerations

- Use of qPCR in cancer diagnostics
- Qualitative and quantitative assessment of Cell free DNA
- PCR for clinical diagnostics and for release of drug products
- Digital PCR in mutation detection and liquid biopsy
- Single cell analysis
- Multiplexing principles and methods
- Different facets of sample preparation (Novel methods of handling/treating sample, isolation, single cell techniques, CTC, sample source, screening/antibody test)
- Importance of understanding if the sample is denatured in anyway
- Using Digital PCR in combination with NGS

Technological Considerations

- What are the recent advances? What are the different approaches?
- Discussion on different platforms, their architecture
- Do new platforms offer highly multiplex analysis?

Data Analysis and Standardisation

- Uniformity of data
- Accuracy and reproducibility of Digital PCR
- Read-out systems
- Methods to analyse multiplex data
- What are the novel bioinformatics tools?
- Importance and necessity of standardisation
- Update on MIQE guidelines

Global Engage is pleased to announce the **3rd Liquid Biopsies Congress**, part of Research & Technology Series will examine the latest biomarker discovery, diagnostic tools and cellular analysis.

This conference offers expert working in the field an opportunity to examine the latest approaches in biomarker detection, circulation tumour cells, precision medication applications, extracellular vesicles and the growing field of single cell analysis.

With 20 speakers from researchers, industry and solution providers, as well as access to the co-located Flow Cytometry Congress and qPCR & Digital PCR Congress, this meeting will allow you to stay up to date with the latest research and explore opportunities on new partnerships in this field.

- Advances in analysing cell free DNA, CTCs, micro-RNA and DNA methylation
- Applying liquid biopsies to studying gene expression and detecting tumour-specific genetic aberrations:
- qPCR, Digital PCR and droplet digital PCR
- Next-Generation Sequencing (NGS) and RNA-Seq
- Standardising ctDNA analysis and understanding release mechanisms: secretion and clearance
- Scaling up: applying liquid biopsies to single cell analysis and cell enrichment
- Liquid biopsy as a cancer screening tool for patient selection: immunoprofiling and detecting resistance mechanisms
- Considerations in for preanalysis in clinical settings
- Liquid biopsies on fluids beyond blood and the potential of non-invasive testing
- Novel cancer biomarkers and companion diagnostics
- Detection of minimal residual disease
- Monitoring treatment response and drug resistance

Global Engage is pleased to announce that the **3rd Flow Cytometry Congress** will be examine the latest biomarker discovery, diagnostic tools and cellular analysis. This highly versatile method is constantly expanding and used in various fields of applications such as oncology, autoimmunity, hematology, infectious disease monitoring and many more, making it important not only in the discovery of new biomarkers, but also in clinical validation and routine implementation. Flow cytometry has allowed unprecedented detail in studies of the immune system and other areas of cell biology with presentations on next big things in flow, cytometry characterisation, machine learning, imaging and more will be discussed at this event.

With 20 speakers from researchers, industry and solution providers, as well as access to the co-located Liquid Biopsies Congress and qPCR & Digital PCR Congress, this meeting will allow you to stay up to date with the latest research and explore opportunities on new partnerships in this field.

- Advancements in fluidic systems: reviewing the latest systems and new approaches in hydrodynamic and acoustic-assisted focussing
- Developing computational analysis and automation
- Approaches to integrating with wider cytometry methods: imaging, mass, and others
- Bead based assays and new approaches to assay development
- Applying flow cytometry to sub-cellular products: RNA and cytokine analysis techniques
- Disease monitoring and patient health: applications in oncology, haematological diseases, and immuno and neuro-inflammation
- Evaluating the use of flow cytometry in the clinic: translational considerations, improving drug design, monitoring drug response
- Integrating with liquid biopsy technology for CTC detection
- Advancing cell sorting and applying it in a clinical environment

Diamond Sponsors

Platinum Sponsors

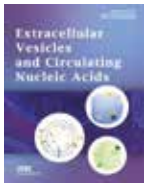
Gold Sponsors

Silver Sponsor

Bronze Sponsor

Exhibitor & Content Sponsors

Media Partner



SPONSORSHIP AND EXHIBITION OPPORTUNITIES AVAILABLE

For sponsorship opportunities contact Gavin Hambrook / Nick Best at:
Sponsorship@globalengage.co.uk or call +44 (0) 1865 849841

qPCR & DIGITAL PCR SPEAKERS

STEPHEN BUSTIN
Professor of Molecular Medicine, Medical Technology Research Centre, Anglia Ruskin University, UK

MIKAEL KUBISTA
Professor, Czech Academy of Sciences, Czech Republic and CEO and Co-Founder, TATAA Biocenter, Sweden

STEPHANIE FRALEY
Associate Professor of Bioengineering, University of California San Diego, USA

KAREN KEMPSELL
Senior Scientist/Project Team Leader, Public Health England, UK

CATHERINE KIBIRIGE
Research Associate, IAVI Human Immunology Laboratory, Imperial College, UK

ALEXANDRA BOGOŽALEC KOŠIR
Research Scientist, National institute of Biology, Slovenia

LILY LI
Viral Vector Analytical Senior Scientist, Cell and Gene Therapy Catapult, UK

NICOLAS YEUNG
Scientist, International Flavors and Fragrances Health and Biosciences, Finland

ANDERS STAHLBERG
Associate Professor and Principal Investigator, Laboratory Medicine, University of Gothenburg & Sahlgrenska University Hospital, Sweden

VALERIE TALY
CNRS Research Director, University of Paris, Centre de Recherche des Cordeliers

DANIEL LOFGREN
Market Development Manager, Digital PCR/PCR, EU, QIAGEN

GURO LIND
Professor, Group Leader Epigenetics, Molecular Oncology Department, Institute for Cancer Research, Oslo University Hospital, Norway

GUILLAUME PAVLOVIC
Head of Unit, Genetic Engineering and Model Validation Department, PHENOMIN-ICS, IGBMC, France

MARINE JEANMOUGIN
Researcher, Molecular Oncology Department, Institute for Cancer Research, Oslo University Hospital, Norway

VIKTOR ADALSTEINSSON
Associate Director, Gerstner Center for Cancer Diagnostics, Broad Institute of MIT and Harvard, USA

SAULIUS KLIMAŠAUSKAS (Chair)
Distinguished Professor, Head of Department, Institute of Biotechnology, Vilnius University, Lithuania

MATTHIEU LEWIS
Market Strategy Manager, Takara Bio Europe

NICHOLAS LESLIE
Professor and Director of Research at the School of Engineering and Physical Sciences, Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, UK

ROSARIO TIZZONE
Regional Sales Specialist Genomics – EMEA, Bio-Rad

RAMON KRANASTER
CEO, myPOLS Biotec GmbH

LIQUID BIOPSIES SPEAKERS

MIKE MAKRIGIORGOS
Professor (Radiation Oncology), Dana Farber and Harvard Medical School, USA

TIM AITMAN
Professor of Molecular Pathology and Genetics, University of Edinburgh

CHRISTA NÖHAMMER
Senior Scientist, Molecular Diagnostics, Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH

EVI LIANIDOU
Professor of Analytical and Clinical Chemistry, National and Kapodistrian, University of Athens, Greece

AN HENDRIX
Professor and Principal Investigator, Ghent University and Cancer Research Institute Ghent

SARAH HÜCKER
Post Doc, Fraunhofer-Institut für Toxikologie und Experimentelle Medizin ITEM-R

CATHERINE ALIX-PANABIÈRES
Director of the Laboratory of Rare Circulating, Human Cells (LCCRH), University Medical, Center of Montpellier, France

JÖRG TOST
Director, Laboratory for Epigenetics and Environment, CEA – National Centre for Research on Human Genomics

FRED RUSSELL KRAMER
Professor of Microbiology, Biochemistry & Molecular Genetics, Rutgers University

BRUNO COSTA-SILVA
EMBO Young Investigator, Head of Systems Oncology Group, Champalimaud Research, Champalimaud Centre for the Unknown

JÓN JÓHANNES JÓNSSON
Professor of Biochemistry, University of Iceland, Medical Director of Genetics and Molecular Medicine, Landspitali, Iceland

ED SCHUURING
Full Professor in Molecular Oncological Pathology, Senior Clinical Scientist in Molecular Pathology, Head of the Laboratoy for Molecular Pathology, University Medical Center Groningen

STEPHANIE JORDAENS
Biomedical Scientist, Novosanis

FLOW CYTOMETRY SPEAKERS

J. PAUL ROBINSON
SVM Professor of Cytomics Purdue University

YU-HWA LO
Professor, University of San Diego

NIMA AGHAEEPOUR
Assistant Professor Stanford University

CHRIS JONES
Associate Professor, University of Reading

HERVE LUCHE
Scientific Director, INSERM

ZIV PORAT
Head of Flow Cytometry Unit, Associate staff scientist, Life Sciences Core Facilities, Weizmann Institute of Science

JOSHUA WELSH
Research Fellow, National Cancer Institute

LEI CHEN
Senior Researcher, University of Sweden

BEN FANCKE
Senior Scientist, NeoGenomics

LUCY WHEELER
Senior BMS Immunology & Immunogenetics, Severn Pathology, Southmead Hospital

MARTIJN VAN BAALEN
Head of the Flow Cytometry Facility, Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital Amsterdam, The Netherlands

SEAN ROONEY
Chief Medical Scientist, Haematology, Our Lady's Hospital for Sick Children

JOHN C TIGGES
Technical Director, Flow Cytometry Science Center, Center for Extracellular Vesicle Research, Beth Israel Deaconess Medical Center

DANIEL PAYNE
Principal Clinical Scientist, Leeds Teaching Hospitals NHS Trust, UK

08:50-09:00 Global Engage Welcome Address

QPCR & DIGITAL PCR

Track Chair: **Valerie Taly**, CNRS Research Director, University of Paris, Centre de Recherche des Cordeliers

09:00-09:40

STEPHEN BUSTIN

Professor of Molecular Medicine, Medical Technology Research Centre, Anglia Ruskin University, UK

Extreme PCR testing for SARS-CoV-2

COVID-19 has provided a comprehensive demonstration of the central role played by molecular diagnostic testing in disease outbreak monitoring and control. A complete testing procedure involves a pre-analysis multistep sample collection, transportation storage and extraction process followed by analysis through reverse transcription, amplification and detection. There are significant drawbacks associated with the current pre-analysis workflow, which uses invasive sampling and may result in variable amounts of extracted viral RNA. Reverse transcription and amplification generally involve protocols that are 30 years old and results in assays taking longer than necessary to complete. We propose streamlining the preanalysis workflow into a single extraction enrichment and RT step followed by extreme PCR to complete a test in less than five minutes.

09:40-10:20

MIKAEL KUBISTA

Professor, Czech Academy of Sciences, Czech Republic and CEO and Co-Founder, TATAA Biocenter, Sweden

Two-Tailed PCR for Precision Diagnostics

We present a highly specific, sensitive and cost-effective system to quantify miRNA, for typing of cell-free DNA in liquid biopsies and for direct blood genotyping based on novel chemistry called Two-tailed PCR. Two-tailed PCR takes advantage of target-specific primers composed of two hemiprobes complementary to two different parts of the target molecule connected by a hairpin structure. The introduction of short hemiprobes that sense the variable sequences confers exceeding sequence specificity while maintaining the very high sensitivity of PCR. Highly similar targets can be distinguished with superior precision irrespectively of the position of the mismatched nucleotide. Further, the target molecule can be very short, making Two-tailed PCR the preferred method for microRNA profiling as well as analysis of rare sequence variants in cell-free DNA and nucleic acids in formalin fixed paraffine embedded (FFPE) tissues. Two-tailed RT-qPCR has a dynamic range of 7 logs and a sensitivity to detect less than ten target miRNA molecules. Two-tailed PCR is readily multiplexed.

LIQUID BIOPSIES

Track Chair: **William Baird**, Director, Global Engage

09:00-09:40

G. MIKE MAKRIGIORGOS

Professor (Radiation Oncology)
Dana Farber and Harvard Medical School, USA

New approaches for efficient detection of hotspot mutations and cancer biomarkers in liquid biopsies

As the potential of liquid biopsies for prognostic, predictive or early cancer detection applications grows, so does the demand for technical advances to accompany the burgeoning range of applications. We present new developments using qPCR, ddPCR and NGS that enable detection of low level mutations or targeted re-sequencing for liquid biopsy applications at a fraction of the current sample size and cost, while retaining sensitivity and specificity.

09:40-10:20

JÖRG TOST

Director, Laboratory for Epigenetics and Environment, CEA – National Centre for Research on Human Genomics

miRNA profiling in extracellular vesicles using high throughput sequencing in human diseases and disease models

Extracellular vesicles (EVs) constitute a heterogeneous group of small membrane coated vesicles including exosomes and microvesicles containing RNA, proteins, metabolites as well as small regulatory RNAs such as miRNAs that constitute an essential component of eukaryotic cell-to-cell communication. The content of EVs is shaped by the cellular environment and actively selected from the content of the original cell. EVs might contribute to the systemic effects of localized diseases and have great potential as biomarker or even for therapeutic interventions. MicroRNA analysis of extracellular vesicles can be confounded by cell-free miRNAs complexed with protein complexes. Proper, but simple isolation of extracellular vesicle content is a pre-requisite for routine clinical implementation. The MiRNA content of extracellular vesicles has been shown to be altered in various diseases. We will present the workflow we have set-up using high-throughput sequencing for the identification and LNA-enhanced qPCR for validation as well as data on the alteration of miRNA expression in extracellular vesicles in different complex diseases.

FLOW CYTOMETRY

Track Chair: **Lucy Wheeler**, Senior BMS Immunology & Immunogenetics, Severn Pathology, Southmead Hospital

09:00-09:40

YU-HWA LO

Professor, University of San Diego

Image-guided FACS and 3D imaging flow cytometer empowered by artificial intelligence

Cell type classification and isolation according to imaging and spatial characteristics, beyond traditional fluorescently labeled biomarkers, enable the development of new biological insight and establishment of connections between phenotypical, morphological, and genomic cell information in normal and diseased states. Here we demonstrate a 2D image-guided cell sorter and a 3D imaging flow cytometer using fast scanning laser excitation sources. Both systems feature a cameraless design, which reconstructs cell images from the temporal readout of photomultiplier tubes. The system architectures support deep learning and artificial intelligence, which enable semi-supervised learning and AI-assisted gating for labelled and label-free cell classification and biomarker discovery.

09:40-10:20

J. PAUL ROBINSON

SVM Professor of Cytomics, Purdue University

The Next Big Things in Flow Cytometry

No technology is a stable entity. They change and adapt to changing demands and if they don't, they die, become redundant and end up as footnotes in history! Flow cytometry has adapted – not as quickly as Covid perhaps, but sufficient to survive for perhaps another 20 years as a highly relevant technology. Flow cytometry has proven to be a critical technology in patient evaluation as the recent Covid pandemic showed. The rapid understanding of the immunology of Covid was highly dependent on flow cytometry. Spectral flow cytometry has much more to offer than traditional polychromatic cytometry. There is now a potential for a 2nd generation spectral technology that will provide many new features. The presentation will discuss the engineering developments in next-generation of technology that will open up new frontiers in biotechnology research and most importantly in both research and clinical diagnostics.

10:20-10:50

NICHOLAS LESLIE

Professor and Director of Research at the School of Engineering and Physical Sciences, Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, UK

Enrichment and detection of DNA sequence variants in blood and tissue: PIK3CA mutations in breast cancer

The analysis of tumour-derived sequence variants in circulating cell free DNA is an increasingly common in the detection, characterisation and monitoring of cancer, frequently referred to as liquid biopsy. However, the low abundance of cell free DNA in blood (a few ng/ml) and the relative abundance of wild-type background sequences over informative variants means that the detection of circulating tumour DNA (ctDNA) is challenging. PIK3CA is one of the two most frequently mutated genes in breast cancers, occurring in 30–40% of cases. Four frequent 'hotspot' PIK3CA mutations (E542K, E545K, H1047R and H1047L) account for 80–90% of all PIK3CA mutations in human malignancies and represent predictive biomarkers for the PI3K inhibitor Alpelisib. Here we develop and evaluate methods to enrich and detect these 4 hotspot PIk3CA mutations, using sensitive digital PCR as a gold-standard reference. A PIK3CA mutation-specific nuclease-based enrichment assay was developed, which combined with a low-cost real-time qPCR detection method, enhances assay detection sensitivity from 5% for E542K and 10% for E545K to 0.6%, and from 5% for H1047R to 0.3%. Moreover, we present a novel flexible prediction method to calculate initial mutant allele frequency in tissue biopsy and blood samples with low mutant fraction. These advancements demonstrated a quick, accurate and simple detection and quantitation of PIK3CA mutations in two breast cancer cohorts (first cohort n=22, second cohort n=25). Hence these simple, versatile and informative workflows could be applicable for routine diagnostic testing where quantitative results are essential, e.g. disease monitoring.

10:20-10:50

BEN FANCKE

Senior Scientist, NeoGenomics

Multicolor Identification of Myeloid Derived Suppressor Cells and Dendritic Cell Subsets in PBMC and Peripheral Blood

Immunotherapy has shown astounding success for the treatment of advanced cancers. Due to the variability seen in patient responses to such therapy there is keen interest to identify cell types responsible for promoting or suppressing anti-tumor immune responses. Dendritic cells (DC) and myeloid derived suppressor cells (MDSC) are leukocytes known to have alternate roles in modulating immune responses, with DC as promoters and MDSC as suppressors of immune function. Each population shows further subdivision within their respective subsets, varying in the capacity to promote or inhibit immune responses. Although differing in their immuno-modulatory roles, DC and MDSC share some common phenotypic characteristics, which complicates the identification of these subsets by flow cytometry. The scarcity of DC/MDSC in peripheral blood and their limited stability ex-vivo creates challenges for the comparison of these populations in a clinical trial setting. Here we define the progression and development of a single multicolor flow cytometry panel for the identification and qualification of all major DC and MDSC subsets and make comparison between the means of collection and handling for the optimization of sample integrity.

10:50-12:00 Morning Refreshments / One-to-One Meetings / Poster Presentations

12:00-12:30

STEPHANIE FRALEY

Associate Professor of Bioengineering, University of California San Diego, USA

High Resolution Melting for Improved dPCR Quantification and Rapid Pathogen Identification

DPCR technology is prized for its capability to accomplish absolute quantification and rare target detection. However, many qPCR best practices that promote assay robustness are not readily applied in dPCR. These include internal amplification controls to account for false-negative reactions and amplicon melt analysis to distinguish true positives from false positives. We have developed a digital high-resolution melting (dHRM) platform to assess the utility of melt-based approaches for mitigation of false positives and false negatives in dPCR. We show that a dHRM-based internal control reduces the inclusion of false-negative partitions, changing the calculated DNA concentration by up to 52%, and enables classification of partitions that would otherwise be considered ambiguous "rain," which accounts for 3%-10% of partitions. When combined with universal primers and machine learning, dHRM technology also accomplished rapid, broad-based pathogen identification.

12:00-12:30

TIM AITMAN

Professor of Molecular Pathology and Genetics, University of Edinburgh

Liquid Biopsy for Easy and Difficult Cancers

Liquid biopsies have started to change the methods of choice for detecting and managing cancer. For cancers that release abundant DNA into the circulation, liquid biopsy is entering routine clinical practice. Our work on head and neck cancer will be described as an exemplar of this. For cancers that are low secretors of cfDNA or where very high sensitivity is required such as in definition of minimal residual disease, I will describe a totally new approach, apheresis-based capture of cfDNA, as a way to increase markedly assay sensitivity.

12:00-12:30

MARTIJN VAN BAALEN

Head of the Flow Cytometry Facility, Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital Amsterdam, The Netherlands

Prepare to explore the tumor micro environment

Biopsies from solid tumors are often small and rare samples. Since this material can only be used once, it's important to obtain a single cell suspension of high quality to probe. In the quest to explore the immune component of the tumor micro environment, preparation and optimization of tissue dissociation are key. The most important aspects in the experimental design phase are covered to obtain high cell yield, viability, and retrieve high quality data from the cells of interest. This presentation has a focus on analysis of immune cells from solid tumors, but the provided information is also applicable to other cellular assays from a wide range of tissue samples.

12:30-13:00

KAREN KEMPSELL

Senior Scientist/Project Team Leader, Public Health England, UK

Challenges in Development of Biomarker Assays for Hard to Diagnose Infectious Diseases

Biomarker-based tests for 'hard to diagnose' conditions have become a key area of interest for development of new diagnostics. 'Omics' technologies and bioinformatics interrogation of large datasets have become an integral part of the biomarker discovery and validation pipeline. Determination of highly specific and sensitive biomarkers for progression can be challenging. Further clinical validation of biomarkers is essential to select the best performing biomarkers, to establish baseline performance and key configurations for multi-biomarker profiles if required. We present work on our diagnostics panels for severe inflammation, sepsis and tuberculosis and discuss their biological context in the inflammatory process and use in diagnostic test development.

12:30-13:00

CHRISTA NÖHAMMER

Senior Scientist, Molecular Diagnostics Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH

Salivary Biomarkers for complex disease diagnostics

The aim of our research activities at AIT, the Austrian Institute of Technology, is to define reliable biomarkers suitable for early and non-invasive complex disease diagnosis and prognosis. To this end we have been establishing and optimizing a whole range of multiplexable technologies (e.g. microarrays, NGS, quantitative PCR, Luminex bead technology) to meet the special demands and challenges of diagnostic biomarker discovery - and validation in body fluids. Using this specific technology expertise we e.g. successfully discovered autoantibody- as well as DNA methylation -based diagnostic marker panels for the big 4 cancer entities (breast, colon, prostate, lung) in serum or plasma. Based on these success stories and the evident advantages of saliva as a diagnostic matrix our recent special interest is to go for saliva diagnostics and to evaluate saliva for its suitability for circulating biomarker-based diagnostics. Along these lines we will show miRNA-, DNA-methylation-, protein – and antibody profiling data demonstrating the great overlap of diverse biomarker classes between cell free saliva and serum/plasma, respectively thereof derived extracellular vesicles (EVs). After reporting about the evaluation of various commercially available strategies for EV isolation from saliva, we will present results of a research project where we are looking for salivary and plasma exosome-derived epigenetic biomarkers for early type 2 diabetes diagnosis and have been testing different small RNA library preparation kits for their use in cell-free body fluids.

12:30-13:00

JOSHUA WELSH

Research Fellow, National Cancer Institute

Utility of flow cytometry for extracellular vesicle analysis

Extracellular vesicles (EVs) are sub-micron lipid spheres derived from cells. Interest in EVs is growing due to their potential applications in translational medicine, therapeutics, as well as their role in basic biology. Progress in the understanding of EVs has been hampered by the lack of equipment and standardization in the EV field. In recent years, the development of highly sensitive commercial flow cytometry platforms, along with societal initiatives, has led to flow cytometry being one of the most informative EV characterization methods. Our work at NIH has developed flow cytometry assays, standardization methods, and software for study of EVs at a single particle level and as part of multiplex analysis techniques. Our methods have allowed EV data to be reported in standard units allowing cross-platform data integration, with the ultimate aim of developing an EV atlas.

13:00-13:30

DANIEL LOFGREN

Market Development Manager, Digital PCR/PCR, EU, QIAGEN

Maximise throughput, flexibility and target detection in digital PCR with QIAGEN's QIAcuity

Digital PCR is not a new technology. Initially, the obstacle to using dPCR as a regular research tool was finding the proper applications for this adaptable technology. This is not the case anymore. Digital PCR has now established itself as a trusted technology in research labs and the pharma biotech industry. Though with the increase in the use of dPCR, new challenges have occurred. Early systems were limited in sample throughput, workflow complexity and turnaround time or the amount of target it could detect simultaneously. The QIAcuity nanoplate-based dPCR system from QIAGEN enables you to overcome these hurdles and transform that experience.

13:30-14:30 Lunch / One-to-One Meetings / Poster Presentations

QPCR & DIGITAL PCR

Track Chair:

14:30-15:00

ALEXANDRA BOGOŽALEC KOŠIR

Research Scientist, National institute of Biology, Slovenia

Validation of multiplex digital PCR for monitoring of infectious diseases – can metrology help?

Infectious diseases are a major burden on national healthcare systems. Bacterial culturing, although most used, is time consuming and can delay introduction of targeted treatment. Though molecular approaches can significantly speed up the pathogen identification process, they also experience measurement challenges. To support rapid molecular diagnosis, we implemented a metrologically guided development, optimization and selection of three species-specific dPCR assays, targeting the most common Gram negative bacteria associated with nosocomial infections and sepsis: Acinetobacter baumannii, Klebisella pneumoniae, and Pseudomonas aeruginosa. As clinical samples may be limited in quantity, simplex assays were combined in one 3-plex assay. Additionally, all four assays were transferred to qPCR, to enable easier implementation in the current clinical setting. Developed assays were characterised in terms of precision, accuracy, sensitivity, and robustness. All dPCR assays were reproducible (CV <25%) on a wide dynamic range, with limits of quantification <100 copies/reaction for 3-plex, and even <50 copies/reaction for simplex assays. All assays proved to be robust as different reaction conditions, as well as transfer to different dPCR and qPCR platforms had no significant effect on detection, or quantification ability.

15:00-15:30

GURO LIND

Professor, Group Leader Epigenetics, Molecular Oncology Department, Institute for Cancer Research, Oslo University Hospital, Norway

Early detection and monitoring of cancer using biomarker-based liquid biopsy analyses

To overcome known challenges tied to lack of accuracy of biomarker based liquid biopsy cancer tests, we have developed an approach where we have combined methylome sequencing data analyses for identification of suitable biomarkers, with robust downstream analyses using our highly standardized droplet digital PCR solution (cf. talk from Dr Jeanmougin). By this approach we have developed a urine-based test for early detection and monitoring of bladder cancer patients for recurrence. In a blinded prospective study the test achieved higher accuracy than current commercially available bladder cancer tests. A national multi-centre trial is ongoing to evaluate

LIQUID BIOPSIES

Track Chair: **William Baird**, Director, Global Engage

14:30-15:30

FRED RUSSELL KRAMER

Professor of Microbiology, Biochemistry & Molecular Genetics, Rutgers University

Sensitive Multiplex SuperSelective PCR Assays for the Quantitation of Rare Somatic Mutations in Liquid Biopsies

SuperSelective primers, by virtue of their unique design, enable the simultaneous identification and quantitation of rare somatic mutations in routine multiplex PCR assays, while virtually eliminating signals from abundant wild-type sequences closely related to the target mutations. These assays are sensitive, specific, rapid, and low-cost, and can be carried out in widely available spectrofluorometric thermal cyclers. As an example, these assays can quantitate seven different somatic EGFR mutations and a wild-type reference gene in the plasma obtained from patients with non-small cell lung cancer. Moreover, the use of pairs of SuperSelective primers for each target prevents false-positive signals. Consequently, multiplex SuperSelective PCR assays of DNA fragments obtained from non-invasive liquid biopsies enable the choice of an effective targeted therapy, the determination of its effectiveness over time, and the substitution of a more appropriate therapy as new mutations arise.

FLOW CYTOMETRY

Track Chair: **Lucy Wheeler**, Senior BMS Immunology & Immunogenetics, Severn Pathology, Southmead Hospital

14:30-15:00

ZIV PORAT

Head of Flow Cytometry Unit, Associate staff scientist, Life Sciences Core Facilities, Weizmann Institute of Science

Utilizing Machine learning for Imaging Flow Cytometry analysis

Imaging Flow Cytometry (IFC) combines the high-throughput quantification of flow cytometry with the high-resolution, information-rich imagery of microscopy. Multi-parametric analysis of image data is highly sophisticated and requires dedicated tools and great expertise. While the data acquisition is usually simple and straight forward, the analysis is the bottleneck for fully utilizing the full power of IFC. The new machine learning (ML) module for analysis of IFC data by AMNIS simplifies this process, as it does not require advanced knowledge of image analysis. In this lecture I will introduce the basic principles of machine learning in image analysis. I will show examples of its usage of in two of our recent projects, the quantification of Golgi fragmentation and analysis of viral factories of the large DNA virus, the Mimivirus.

15:00-15:30

HERVE LUCHE

Scientific Director, INSERM

Immuno-Profiling of Murine preclinical Tumor Models in the Cytomic area: A way to improve Translational Prediction for Immune-Based Therapeutics

The identification of the major cellular players involved in the progression of a type of cancer is a key step for the success of new immunotherapies for effective personalized medicine. It is however a daunting challenge because complex relationships exist between tumor cells and immune system cells. To characterize the impact of a gene involved in the anti-tumor response, cellular phenotyping of leukocytes infiltrating a tumor but also those present in peripheral organs is necessary. Flow cytometry describing cellular heterogeneity does not evaluate the effector functions of immune cells. Mass cytometry is the sampling technique of choice as it is amenable for the simultaneous detection of surface markers but also functional

15:00-15:30

the clinical utility of the test for surveillance of recurrence among bladder cancer patients. We also demonstrate that ddPCR DNA methylation analyses of small volumes of bile samples (200ul) can be used for early and accurate detection of the deadly bile duct cancer in high risk individuals.

15:00-15:30

Continued

15:00-15:30

markers (cytokines, transcription factors) at the single cell level. We study a set of these markers at the level of the single cell in mice grafted with MC38 tumors treated or not by an immuno-oncological agent. For explorational studies, scGen approaches (CITEseq) of genomic approaches may also be integrated in the immune-profiling pipeline. Through this presentation, I will show the route we followed to build the phenotyping engine of CIPHE both in terms of application as well as analysis. Application on pre-clinical characterization of new therapeutics or refined preclinical models (humanized mice) will be presented.

15:30-15:45

MATTHIEU LEWIS

Market Strategy Manager, Takara Bio Europe

How Research Tools and Enzymes Became Fundamental Components of Diagnostic Solutions for SARS-CoV-2

Takara Bio is a global biotechnology company that has decades of experience in the research and development of high-quality enzymes and solutions for DNA and/or RNA based molecular diagnostics. Recently, active collaboration with scientists around Europe and with Takara Bio's R&D has allowed to develop faster and more efficient protocols for the detection of SARS-CoV-2, with an eye for the post-Covid19 era where PCR technology will be democratised and continuously used. During this presentation, we will present how the use of research tools and enzymes became fundamental components of molecular diagnostic solutions and how the scientific community played a critical role in making disease testing more streamlined and more powerful.

15:30-15:45

STEPHANIE JORDAENS

Biomedical Scientist, Novosanis

Urine, a "golden sample" for cancer biomarker testing

Urine as a non-invasive liquid biopsy offers huge potential for cancer biomarkers testing. Several diagnostic assays using urine are commercially available or on the verge of launch in clinical practice. We believe urine has the potential to become the "golden sample" for cancer biomarker testing, enabling cancer detection and monitoring in a non-invasive and easy way. Several studies have shown the potential of urine as a non-invasive liquid biopsy due to the ease of sampling and high acceptability compared to blood and tissue. Indeed, a standard urine cup has limitations and can be awkward, messy, and inconvenient for the user. First-void urine, the first 20 – 30 mL of urine flow, has shown to contain valuable biomarkers for several cancer types. Colli-Pee® by Novosanis allows for standardized and volumetric first-void urine collection and stabilization. The device is available in different volumes depending on the application. In addition, the device architecture enables immediate mixing with a stabilization chemistry. This can improve sample collection for downstream analysis by ensuring analytes of interest are preserved for laboratory assay. Colli-Pee® is available neat and prefilled with the stabilizer UCM, allowing the preservation of DNA in urine. Some variants are CE-IVD marked, and registered in several countries outside of Europe.

15:30-15:45

No Track Talk

15:45-16:35

Afternoon Refreshments / One-to-One Meetings / Poster Presentations

16:35-17:05

CATHERINE KIBIRIGE

Research Associate, IAVI Human Immunology Laboratory, Imperial College, UK

Screening HIV-1 Vaccine and Cure-Therapy Candidates by Quantifying CD4 T Cell Viral Nucleic Acid Levels and Integration Site Profiles

A vaccine or cure for HIV-1 has eluded scientists due to the high mutation rate of the virus and the persistence of a latent reservoir. The viral inhibition assay (VIA) is an in vitro tool assessing functional CD8 T cell responses. The VIA assesses vaccine

16:35-17:05

AN HENDRIX

Professor and Principal Investigator, Ghent University and Cancer Research Institute Ghent

Methodological considerations to study extracellular vesicles in liquid biopsies

The identification of extracellular vesicle (EV)-associated biomarkers is challenging owing to the complexity of liquid biopsies (PMID: 33568799). We 1) performed quality control studies to identify the impact of (pre-) analytical variables on biomarker identification (PMID: 31776460; PMID: 33111109;

16:35-17:55

WORKSHOP:

JOHN C TIGGES

Technical Director, Flow Cytometry Science Center, Center for Extracellular Vesicle Research, Beth Israel Deaconess Medical Center

CytoFLEX SRT: The CytoFLEX That Sorts

The CytoFLEX SRT Is a compact cell sorter capable of handling the variety of samples that are presented to a core facility. From bacteria to tumor cells, and everything in between, the SRT allows a core facility the diversity it requires to assist the entire

16:35-17:05

candidates and correlates with in vivo virus control. A rapid PCR-based version of the assay could provide a more efficient, high throughput tool for screening vaccine candidates. A rapid robust biomarker to evaluate HIV cure-therapy candidates is still required. I hypothesize that the integration site profiles of HIV in CD4+ Th17 cells, including integration sites and clonal sizes, could be used as a biomarker of treatment outcome. I present the rationale for this work including our most recent data.

16:35-17:05

PMID: 32284825;), 2) developed reference materials to ensure standardized EV measurements (PMID: 31337761; PMID: 33452501), and 3) created EV-TRACK to stimulate researchers to put experimental guidelines into practice (PMID: 28245209). This combined expertise boosted the identification of bacterial EVs in the systemic circulation of patients with intestinal barrier dysfunction (PMID: 30518529).

16:35-17:55

research community. In this workshop, individuals will hear from Flow Cytometry Core members at Beth Israel Deaconess Medical Center about the CytoFLEX SRT's use in a multi-user setting. The following topics will be presented and discussed:

- Ease of instrument use, including start-up and shutdown.
- Comparison of CytoFLEX SRT and CytoFLEX S.
- Common applications for SRT sorting.
- Novel applications using the SRT.

17:05-17:55

WORKSHOP:
MIKAEL KUBISTA

TATAA BIOCENTER

Professor, Czech Academy of Sciences, Czech Republic and CEO and Co-Founder, TATAA Biocenter, Sweden

The MIQE Guidelines: How to get the most out of your molecular measurements

- Control the whole workflow from sampling to data analysis
- Work according to the MIQE and dMIQE guidelines and the new Technical Specifications and Standards for Molecular in vitro diagnostic examinations by the European Committee for standardization (CEN) and the International Organization for Standardization (ISO)
- Sampling, storage, transportation and isolation of nucleic acids and proteins from biofluids and tissue samples
- Procedures and protocols for RNA, DNA and protein isolation
- Quality assessment of RNA, DNA and proteins

Why MIQE guidelines?
The MIQE and dMIQE guidelines help researchers presenting qPCR and dPCR results in scientific publications and teach reviewers what to request in quality manuscripts.

Why Quality Control of the Pre-analytical phase?
The sampling, handling and storage of samples affects all downstream applications and the final analysis result. Within the European projects SPIDIA(4P), the pre-analytical phase was studied and factors impacting the analytical test results were identified. It was concluded that in many processes the dominant contribution to the total error in the testing process is from the pre-analytical phase.

Why CEN/ISO Technical Specifications and standards?
These specifications were produced to achieve reliable and robust sample handling and preparation in a standardized way to ensure measured data are of high quality and comparable across laboratories. The new guidelines are particularly relevant for accredited laboratories but are also valuable for research, where procedures are standardized, and data compared. The workshop introduces the new Technical Specifications and guidelines and brings them into context for smooth implementation in routine laboratory procedures.

GenEx software
All course participants will receive 3 months license for the GenEx software used in workshop exercises to validate and analyze data.

Who should attend?

- Quality managers at medical, diagnostic and research laboratories
- Laboratory personnel at medical, diagnostic labs and research laboratories
- Biobank personnel
- Managers and researchers coordinating or performing biomarker studies and trials

Registrants are required to bring your own laptop for software exercises during the session.

17:55 End of Day 1 / Networking Drinks Reception

08:50-09:00 Global Engage Welcome Address

QPCR & DIGITAL PCR

Track Chair: **Saulius Klimasauskas**, Distinguished Professor, Head of Department, Institute of Biotechnology, Vilnius University, Lithuania

09:00-09:40

ANDERS STAHLBERG

Associate Professor and Principal Investigator, Laboratory Medicine, University of Gothenburg & Sahlgrenska University Hospital, Sweden

Preamplification strategies for qPCR and dPCR

Analyses of multiple DNA and RNA targets in limited sample sizes, such as liquid biopsies, often requires preamplification. We have studied several experimental parameters in targeted preamplification and their effects on downstream qPCR and dPCR, including degree of multiplexing, cycles of amplification, primer concentrations, temperature profiles, polymerase fidelity and additives. The goal of preamplification is to generate sufficient amount of target molecules to allow singleplex quantification of multiple targets in a reproducible, specific, and sensitive manner. As this may require target sequences to be multiplied several orders of magnitude, subsequent sample handling becomes a potential contamination hazard. Here, we show how the use of dUTP combined with heat-sensitive uracil-DNA N-glycosylase minimizes the risk of contaminations. On the basis of our findings, we provide recommendations how to perform robust and highly accurate targeted preamplification in combination with qPCR and dPCR

09:40-10:20

VALERIE TALY

CNRS Research Director, University of Paris, Centre de Recherche des Cordeliers

Digital PCR for cancer patient follow-up

Droplet-based digital PCR allows for unprecedented sensitivity and accuracy for rare sequences detection and quantification including genetic and epigenetic tumor-specific alterations. We will discuss how ddPCR assays could be set-up to highlight presence of ctDNA in several clinical contexts both as a "stand-alone" assay but also in complement to other technologies such as optimized high sensitivity Next Generation Sequencing (NGS). We will illustrate their pertinence for overcoming clinical oncology challenges by presenting the results of different prospective studies.

LIQUID BIOPSIES

Track Chair: **William Baird**, Director, Global Engage

09:00-09:40

ED SCHUURING

Full Professor in Molecular Oncological Pathology, Senior Clinical Scientist in Molecular Pathology, Head of the Laboratoy for Molecular Pathology, University Medical Center Groningen

Circulating tumor DNA as an early on-treatment predictive biomarker for patients with advanced non-small cell lung cancer receiving immune checkpoint inhibitors

- Circulating tumor DNA (ctDNA) is shed into the bloodstream and serves as potential predictive or prognostic biomarker
- Changes in mutant ctDNA levels in plasma cfDNA detected with ddPCR or ctNGS are associated with overall survival in NSCLC patients treated with immune checkpoint inhibitors
- Interpretation of ctDNA NGS results needs to account for clonal hematopoiesis of indeterminate potential (CHIP) representing variants in cfDNA unrelated to the lung cancer

09:40-10:20

CATHERINE ALIX-PANABIÈRES

Director of the Laboratory of Rare Circulating Human Cells (LCCRH), University Medical Center of Montpellier, France

Metastasis-Competent Circulating Tumor Cells in Colon Cancer

The development of blood-based, tumor-specific biomarkers called real-time liquid biopsy such as circulating tumor cells (CTCs) have made significant advances over the last years in cancer research. Real-time liquid biopsy has been introduced as a new diagnostic concept predicated on the analysis of CTCs or circulating tumour-derived factors. Highly sensitive liquid biopsy assays have been developed. As an in-depth investigation of CTCs is hampered by the very low number of these cells, especially in the blood of colorectal cancer patients, the establishment of cell cultures and permanent cell lines from CTCs has become the most challenging task over the past year. In my talk, I will describe our work on the in vitro expansion of colon CTCs from a metastatic colon cancer patient and all the information that we could get from this precious and unique biological material. The establishment of CTC lines represents a new opportunity to decipher the metastatic cascade and, hopefully, to find ways to stop.

FLOW CYTOMETRY

Track Chair: **Christopher Jones**, Associate Professor, University of Reading

09:00-09:40

SEAN ROONEY

Chief Medical Scientist, Chief Medical Scientist, Haematology, Our Lady's Hospital for Sick Children

Rare Event Analysis in Clinical Flow Cytometry

'Rare event' acquisition and analysis by Flow Cytometry has increasingly become an essential tool for the identification, characterisation and monitoring of a wide range of Haematological malignancies. Particularly useful is the ability to detect very low levels of disease (0.01%) in patients who have completed various stages of treatment (MRD Minimum Residual Disease). The quantitation of these low levels of disease has been shown to correlate with survival rates and are being used to alter patient's treatment protocols. This is resulting in improved outcomes across a range of cancers. There are a number of technical issues that must be considered to ensure precise and consistent quality of flow cytometric results. These will be discussed under the following headings, sample quality, antibody/fluorochrome selection, instrument set up and data analysis. These will be illustrated with clinical cases.

09:40-10:20

NIMA AGHAEEPOUR

Assistant Professor, Stanford University

Machine Learning for Multiomics Analysis of the Immune System

Recent technological advances in science provide novel opportunities to unravel the complex biology of diseases. Immunological changes in translational settings are often highly dynamic and involve multiple interconnected biological systems. We will discuss a series of machine learning innovations which enable objective analysis of single-cell immunologic data robust to small variations in patient cohorts, as well as integration with prior knowledge to increase predictive power without increasing cohort size. Next, we will discuss integration of single cell data into a multiomics setting using a customized machine learning algorithm. This computational pipeline increases predictive power and reveals new biology, by combining datasets of various sizes and modularities in a balanced manner.

10:20-10:50

ROSARIO TIZZONE

Regional Sales Specialist Genomics – EMEA, Bio-Rad

BIO-RAD

Robust and reproducible quality control in your Cell & Gene Therapy development with ddPCR

Recent rapid advances in techniques like CRISPR gene editing and gene-modified cell therapies such as chimeric antigen receptor (CAR) T cells promise to deliver solutions to diseases that were previously considered untreatable. However, developing effective and reproducible gene and cell therapies requires the use of sensitive and robust testing methods to validate the quality as well as safety and efficacy of the therapeutic product. Here we will look at some of the most common challenges facing developers of these therapies, including low vector concentration, empty capsids, contaminating host cell DNA or mycoplasmas and how Bio-Rad's portfolio of Droplet Digital PCR (ddPCR) solutions are being used throughout the gene or cell therapy development process to overcome these challenges and provide a robust and reproducible quality control.

10:50-12:00 Morning Refreshments / One-to-One Meetings / Poster Presentations

12:00-12:30

GUILLAUME PAVLOVIC

Head of Unit, Genetic Engineering and Model Validation Department, PHENOMIN-ICS, IGBMC, France

Evaluation of the reliability of droplet digital PCR and RT-ddPCR on a large number of samples and targets

Droplet digital PCR (ddPCR) is a powerful tool for the precise quantification of DNA or mRNA targets. Compared with qPCR, ddPCR is less sensitive to technical bias, but good protocols and a broad understanding of the technical limitations remain essential to ensure the quality of published results and to avoid experimental nonsense data. In the last years, we analyzed thousands of samples and hundreds of target genes from genetically altered mouse lines using ddPCR or RT-ddPCR. Here, we present our protocol for DNA, RNA, or simultaneous DNA/RNA studies validated on this large dataset including multiple tissues. We will discuss some ddPCR and RT-ddPCR technical optimizations and limitations. Finally, we will present some recommendations on assay design and data analysis.

12:00-12:30

EVI LIANIDOU

Professor of Analytical and Clinical Chemistry National and Kapodistrian University of Athens, Greece

Molecular assays for a comprehensive liquid biopsy approach

12:00-12:30

LUCY WHEELER

Senior BMS Immunology & Immunogenetics, Severn Pathology, Southmead Hospital

The establishment of a UK wide ALL Network

- Challenges around the standardisation
- Challenges involved with ALL MRD
- Case studies about the use of novel therapies.

12:30-13:00

MARINE JEANMOUGIN

Researcher, Molecular Oncology Department, Institute for Cancer Research, Oslo University Hospital, Norway

PoDCall - Positive droplet calling and normalization of droplet digital PCR methylation data

Droplet digital PCR (ddPCR) has great potential for DNA methylation analyses. However, the lack of consensus regarding how to perform standardized methylation-specific ddPCR experiments is challenging. To increase the accuracy of ddPCR DNA methylation analyses we have developed PoDCall, a software that performs both (i) automated positive droplet calling and (ii) normalization of ddPCR data using the 4plex, a robust internal control developed in-house. PoDCall has been extensively evaluated in cell lines and successfully used for early detection of bladder cancer and cholangiocarcinoma, in urine and bile samples respectively (cf. talk from Prof. Lind).

12:30-13:00

BRUNO COSTA-SILVA

EMBO Young Investigator, Head of Systems Oncology Group Champalimaud Research, Champalimaud Centre for the Unknown

Extracellular Vesicles as emerging players in cancer biology and diagnostic applications

Extracellular Vesicles (EVs), membrane vesicles released by all cells, are emerging mediators of cell-cell communication. By carrying biomolecules from tissues to biofluids, EVs have attracted attention as non-invasive sources of clinical biomarkers in liquid biopsies. We here present a Flow Cytometry (FC) strategy that reduce biofluids processing time, costs and volume requirements by not requiring isolation or concentration of EVs prior to staining. We illustrate its application to monitor tumor-associated EVs populations in metastatic pancreatic cancer patients. We also show unpublished work on the identification of EVs markers of liver metastatic stroma in pancreatic cancers.

12:30-13:00

LEI CHEN

Senior Researcher, University of Sweden

A ultra sensitive targeted mutation detection approach using flow cytometry readout

The ability to observe, evaluate, and count even extremely rare nucleic acid sequence variants in biological samples is a frequent need in biology and medicine. In particular, rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy in clinical routine, but improved assay techniques are needed for broad adoption. We describe herein a methodology - SafeLock assays - which provides for rapid and highly specific detection of exceedingly rare DNA sequence variants. We demonstrate precise, ultra-sensitive detection of single-nucleotide mutant sequences from malignant cells against a 100,000-fold excess of DNA from normal cells in either bone marrow or peripheral blood to

12:30-13:00

PoDCall was compared to QuantaSoft, the software provided with the Bio-Rad's QX200™ Droplet Digital™ PCR System and showed good performance in all settings. The shiny application allows users to perform analyses in an interactive visual environment and thresholds can also be visually inspected and manually corrected.

12:30-13:00

Continued

12:30-13:00

follow the course of patients treated for acute myeloid leukemia (AML). Also rare sequence variants located in mutation-prone high-GC regions are sensitively detected. Mutant gene copies remaining after therapy are counted via flow cytometry, and we demonstrate the potential for early detection of disease recurrence, allowing prompt change of therapy.

13:00-13:15

RAMON KRANASTER

CEO, myPOLS Biotec GmbH

DNA polymerases - key parts in most molecular diagnostic applications

The focus of myPOLS Biotec is on the research, development and distribution of DNA polymerase-based systems. DNA polymerases are the key components in many modern diagnostic procedures. For many examinations of blood samples, the laboratory doctor usually has to carry out elaborate DNA isolation before analysis of the existing DNA in the sample. The same is expected for the detection of RNA samples in clinical specimens. myPOLS Biotec demonstrates that it is possible to work without this previous DNA or RNA isolations. This significantly reduces the time required and eliminates numerous error-prone work steps altogether. myPOLS Biotec contributes through the innovation and development of new DNA polymerases, to reducing health and economic costs. myPOLS Biotec continues its vision of making early-diagnostic tests and procedures more cost-effective, faster and thus more versatile. In the lecture Dr. Ramon Kranaster (CEO of myPOLS Biotec) will present the latest results from R&D activities.

13:15-14:15 Networking Lunch / One-to-One Meetings / Poster Presentations

QPCR & DIGITAL PCR

Track Chair: **Valerie Taly**, CNRS Research Director, University of Paris, Centre de Recherche des Cordeliers

14:15-14:45

VIKTOR ADALSTEINSSON

Associate Director, Gerstner Center for Cancer Diagnostics, Broad Institute of MIT and Harvard, USA

Ultrasensitive detection of minimal residual disease

Liquid biopsies could enable cancer treatment response monitoring but have limited sensitivity to detect minimal residual disease. Here, I will describe our team's efforts to increase the sensitivity of liquid biopsies to detect circulating tumor DNA at 1 to 10 parts-per-million. I will present new technologies for mutation enrichment and high-accuracy sequencing and provide proof-of-principle application to small clinical studies.

LIQUID BIOPSIES

Track Chair: **William Baird**, Director, Global Engage

14:15-14:45

SARAH HÜCKER

Post Doc, Fraunhofer-Institut für Toxikologie und Experimentelle Medizin ITEM-R

Single cell microRNA sequencing

MicroRNA signatures present promising prognostic or diagnostic biomarkers in diseases like cancer. However, until recently only bulk miRNA sequencing was feasible, which already shows biased results because of sequence-sensitive adapter ligation and formation of adapter dimers. On single cell level, only very few protocols are published and no comprehensive protocol comparison was performed. First, we tested 19 different miRNA-Seq protocol variants to systematically investigate their sensitivity and accuracy using single MCF7 cells spiked with 1 pg miRXplore (1,000 miRNAs in equimolar concentration). Second, the eight best performing protocols were selected for miRNA-Seq of MCF7 single cell equivalents. All protocols showed reduced performance on single cell level compared to the spike-in experiment: Adapter dimer reads increased and reads mapping annotated miRNAs decreased. However, 25-300 different miRNAs were detected per cell. Third, the best performing protocol was applied to single cells of eight different cell lines. Finally, we applied miRNA-Seq to clinical samples: Circulating tumor cells were isolated from the blood

FLOW CYTOMETRY

Track Chair: **Christopher Jones**, Associate Professor, University of Reading

14:15-14:45

CHRIS JONES

Associate Professor, University of Reading

Platelet activation dynamics determine thrombus size and structure at arterial but not venous shear

Platelet response to activating stimuli and pharmaceutical agents varies greatly within the normal population. The majority of data on platelet function comes from endpoint assays, yet platelets function in a dynamic environment and the kinetics of their response is likely to be just as physiologically relevant. To evaluate this, we have developed a bespoke real-time flow cytometry assay and an analysis package that enables measurement of the rate of platelet activation over time. The kinetics of platelet activation we assessed in 143 fasted, healthy, aspirin free donors. A recall of 12 individuals from the initial cohort was used to assess the effect of platelet response kinetics on thrombus formation and structure. The rate of platelet activation varied considerably within the normal population, but did not correlate with maximal platelet activation, demonstrating that platelet rate is a separate and novel metric to describe platelet reactivity. The relative rate of platelet response between agonists was strongly correlated, suggesting a central control mechanism regulates the rate of platelet response to all agonists. Furthermore, platelet response

14:15-14:45

Continued

14:15-14:45

of seven lung cancer patients. The expression patterns of known oncogenic miRNAs were patient specific and CTCs of the same patient showed high heterogeneity. Even the identification of novel candidate miRNAs was feasible.

14:15-14:45

kinetics correspond to thrombus size and structure, where faster responders form larger, more densely packed thrombi at arterial, but crucially not venous shear. We have demonstrated that the rate of platelet activation is an important metric in stratifying individual platelet responses. This provides a novel focus for the design and development of anti-platelet therapy, targeting high shear thrombosis without exacerbating bleeding at low shear.

14:45-15:15

NICOLAS YEUNG

Scientist, International Flavors and Fragrances Health and Biosciences, Finland

Moving away from bacterial plate counting using ddPCR

The global interest in probiotics has grown in recent years. Probiotics' health benefits depend on an amount dosed in CFUs (colony forming units). Currently CFUs are still measured using agar plate counting. This has two inherent disadvantages: the first is the error rate of plate count (which can range from 10-30%, necessitating many technical replicates), the second is specificity. Mixing different carbon sources and antibiotics can only separate bacteria at the genus level. Combining ddPCR with extracellular DNA blocking blocking dye can be a path forward to solving both of these issues, giving both a higher precision and strain-level detection.

14:45-15:15

JÓN JÓHANNES JÓNSSON

Professor of Biochemistry, University of Iceland Medical Director of Genetics and Molecular Medicine, Landspitali, Iceland

Extensive structural damage in cfDNA in body fluids

Northern Lights Assay (NLA) in microgels can detect various types of DNA damage including single-stranded breaks, double-stranded breaks, intrastrand and interstrand DNA crosslinks (ICL), single-stranded DNA and bulky lesions in cfDNA in body fluids. DNA is isolated with gentle methods. We tested samples isolated from whole blood, plasma, saliva, urine sediment and cell-free urine taken from healthy subjects and patients with diseases where damage to cfDNA might be expected. cfDNA samples from each body fluid showed patterns that were variable between healthy individuals, but distinctive for each type of fluid. cfDNA from plasma had variable apoptosis patterns. cfDNA in salvia had extensive damage, and single-stranded breaks were very prominent. cfDNA in cell-free urine showed predominantly a necrosis pattern. Blood cell DNA had minimal DNA damage in healthy individuals, but DNA from urinary sediment cells had a combination of apoptosis and necrosis pattern. cfDNA from sepsis patients had specific damage patterns and platinum-treated cancer patients had detectable interstrand crosslinks. NLA of cfDNA isolated from various body fluids might have diagnostic value and used as a companion diagnostic.

14:45-15:15

DANIEL PAYNE

Principal Clinical Scientist, Leeds Teaching Hospitals NHS Trust, UK

Integrated haematological cancer diagnosis and MRD

- Improving the consistency and accuracy of diagnosis has been identified as the single most important aspect of improving outcomes in haematological cancer and forms the basis of NICE Improving Outcomes Guidance.
- Accurate diagnosis of haematological malignancies involves integration of clinical information with several diagnostic modalities.
- HMDS is a multi-disciplinary laboratory service specialising in the diagnosis of haematological cancers, processing 40,000 test requests per year and acting as a diagnostic reference centre for national and international clinical trials.
- Accurate, precise and sensitive monitoring of residual disease is also critical, as this is associated with targeted therapy and improved survival.
- Assessment of residual disease requires consensus best practice, harmonisation and standardisation
- Flow cytometric and molecular methods utilised and in development for MRD assessment will be discussed along with the benefits and challenges of an integrated diagnostic approach for MRD reporting.

15:15-15:45

LILY LI

Viral Vector Analytical Senior Scientist, Cell and Gene Therapy Catapult, UK

Development of qPCR and ddPCR for viral genome titration in AAV gene therapies

For AAV gene therapies, determining the correct AAV clinical dosage – based on viral genome (vG) titre – is pivotal to product safety. qPCR is the standard method in vG titration; however, it displays high variability linked to multiple factors including interference at the amplification step and reliance on a standard curve generation. Both can be overcome using the ddPCR platform. At CGT Catapult we performed a comparability study using both ddPCR and qPCR, and found that the ddPCR is more precise in vG generation. Additionally, we are developing our qPCR and ddPCR to improve accuracy, precision and robustness. Fit-for-purpose vG titration will advance analytical characterisation of process development and manufacturing, ultimately having a direct impact on the safety of dosing AAV therapies for preclinical and clinical studies.

15:45 Conference Close

DON'T DELAY, BOOK YOUR PLACE TODAY!

PHONE BOOKING

+44 (0) 1865 671957 ext. 207

maria@globalengage.co.uk

Our conference team will make all the necessary arrangements.

ONLINE BOOKING

Visit the website to book your place

Flow Cytometry Congress: www.global-engage.com/event/flow-cytometry-congress

qPCR & Digital PCR Congress: www.global-engage.com/event/qpcr-digital-pcr

Liquid Biopsies Congress: www.global-engage.com/event/liquid-biopsies

THE CONGRESS PACKAGE INCLUDES:

Track hop all conference sessions for all collocated meetings
Watch presentations on-demand for two weeks
Give a poster presentation
Join networking discussion rooms
Priority networking with other attendees
Create post-event connections
Conference workbook

FREE NEWSLETTER

For updates on the Research & Technology Series 2021, plus free resources and reports, as and when our speakers authorise their release dates, check for updates at:

Flow Cytometry Congress: www.global-engage.com/event/flow-cytometry-congress

qPCR & Digital PCR Congress: www.global-engage.com/event/qpcr-digital-pcr

Liquid Biopsies Congress: www.global-engage.com/event/liquid-biopsies

SPONSORSHIP AND EXHIBITION OPPORTUNITIES AVAILABLE

For more details contact Nick Best

sponsorship@globalengage.co.uk or call **+44 (0) 1865 849841**

T: +44 (0) 1865 849841
E: info@globalengage.co.uk
www.global-engage.com

Global Engage Ltd., The Kidlington Centre, Kidlington, Oxford, OX5 2DL, United Kingdom

Follow Us