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# 4BIO Summit: Europe

## Poster Presentation Abstracts

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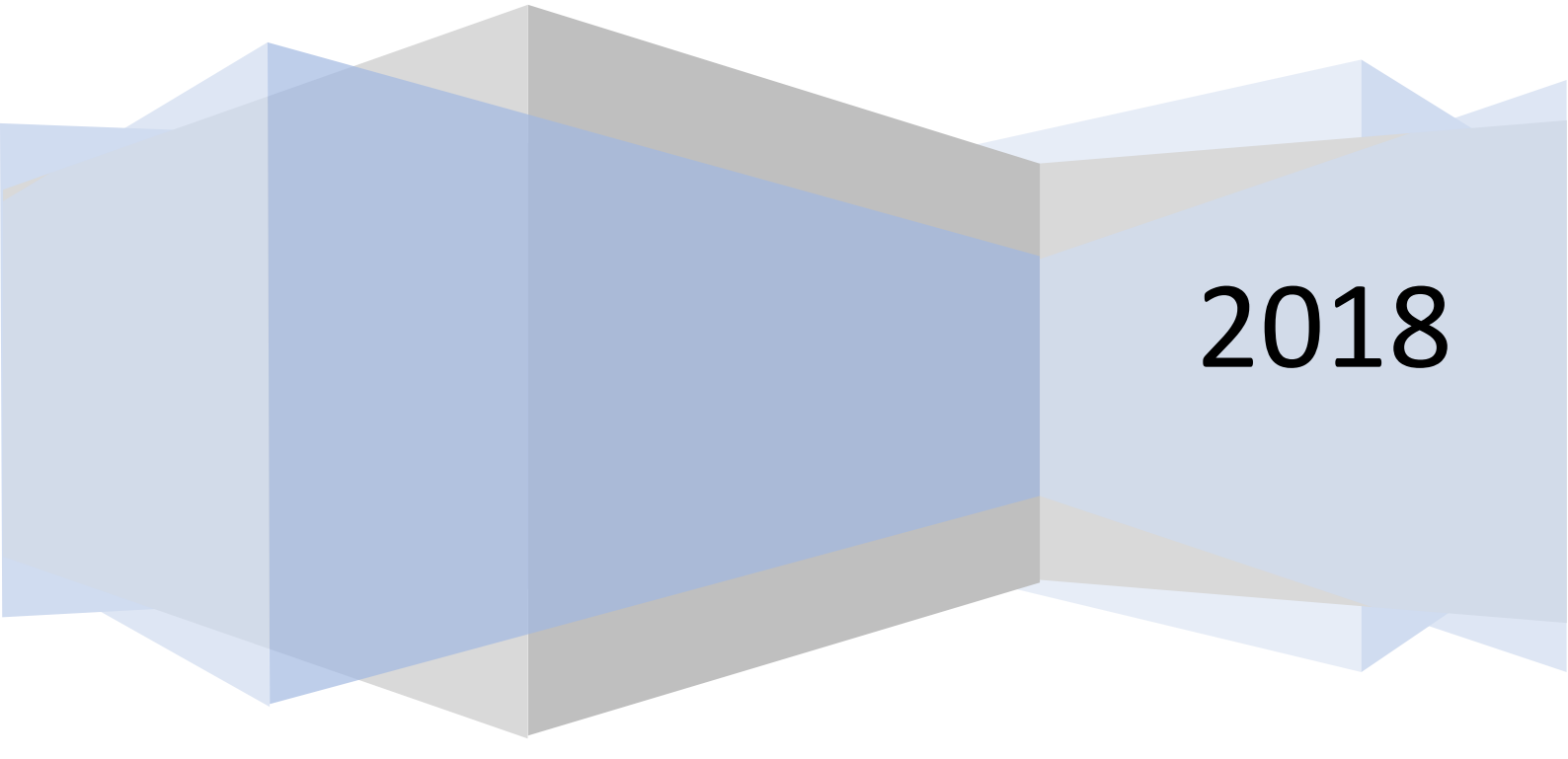

2018

# Poster Titles:

| | Title | Principal Author(s) | Affiliation |
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| 17 | USE OF DIGITAL PCR (dPCR) FOR QUANTIFICATION OF GENETICALLY MODIFIED ORGANISMS (GMO) IN COMPLEX SAMPLES | Alexandra Bogožalec Košir[1,2], Dejan Štebih[1], Tina Demšar[1], Jana Žel[1], Mojca Milavec[1] | [1]National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, 1000 Ljubljana<br>[2]Biotechnical Faculty, Department of Biology, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana |
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| Poster Title | qPCR vs dPCR – Designing a Strategy for Detection and Quantification of Ganciclovir Resistant HCMV |
| Abstract | Methods for fast, accurate and sensitive detection, identification and quantification of pathogens are of paramount importance for reliable and effective disease control. Cytomegaloviruses are evolutionarily ancient viruses that belong to the herpesvirus family. Human cytomegalovirus (HCMV) or human herpesvirus 5 (HHV5) is a 230 kB double stranded DNA virus infecting monocytes, macrophages and dendritic cells. HCMV often leads to wide spread of viral replication and dissemination to multiple organs and can be life threatening to individuals with compromised or immature immune system.<br>Antiviral drugs ganciclovir (GCV), cidofovir and foscarnet are used to treat patients and prevent further dissemination of the virus. Of these the most widely used is GCV, which targets UL54-gene encoding for viral DNA polymerase. As GCV needs to be phosphorylated by phosphotransferase UL97, most resistant mutations occur in the UL97 region. These point mutations or single nucleotide polymorphisms (SNPs) represent 80% of all GCV resistance. As the development of resistance can be life-threatening especially for immunocompromised patients, a rapid method for detection and monitoring of infection and viral load is necessary.<br>Digital PCR (dPCR) is a prosperous methodology that has already been used for many different applications including viral quantification. On the other hand, qPCR remains the method of choice for clinical diagnostics. The aim of our study was to select the most common SNPs that contribute to GCV resistance and design Taq-Man chemistry-based assays for their detection and quantification. These assays would then be assessed on a dPCR (QX100/200, BioRad) and qPCR (Viia7, Life Technologies) platform in order to determine the fitness for purpose of different strategies.<br><br>This research was financially supported by the AntiMicroResist project (the EMRP project, which is jointly funded by the EMRP participating countries within EURAMET and the European Union). |

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| Poster Title | DNA-based testing on cyst fluid to distinguish between pancreatic cyst types |
| Abstract | **Background**<br>Differentiating harmless from potentially malignant pancreatic cysts is critically important to prevent malignant evolution, while also preventing unnecessary surgery. Current methods are suboptimal. DNA-based testing of cyst fluid, obtained by endoscopic ultrasound with fine needle aspiration (EUS-FNA), is emerging to a promising diagnostics tool. Sequencing studies identified distinct mutational profiles for the various cyst types.<br><br>**Aim**<br>To assess the additional value of DNA-based testing of cyst fluid for the distinction between pancreatic cyst types using (*i*) targeted mutational analysis (Ion Torrent Next Generation Sequencing, NGS) and (*ii*) digital droplet PCR (ddPCR).<br><br>**Patients and methods**<br>We included 24 patients who underwent EUS-FNA for pancreatic cysts and surgery (2007-2014). DNA was isolated from cyst fluid and corresponding cyst wall. Custom AmpliSeq panels were designed, and ibraries were run on the Ion Proton™. The same DNA from 7 cyst walls and 24 cyst fluids was used for BioRad XT-100 ddPCR™ using the KRAS G12/G13 Screening Kit.<br><br>**Results**<br>From 42% of cyst fluid samples we isolated sufficient DNA for Ion Torrent targeted sequencing. In 38% of these runs a mutation supporting the diagnosis was identified. Combined with the findings from cyst wall DNA, we predicted the correct diagnosis in 65% of runs, 45% of cases.<br>Using ddPCR, we correctly identified KRAS mutations in 18 cyst fluid samples (75%), and absence in 3 samples (13%). There were 3 (13%) false negative samples. In cyst wall DNA 1 false positive result was observed. These numbers are dependent on the thresholds set for a successful run.<br><br>**Discussion**<br>The amount of DNA that can be extracted from cyst fluid is sufficient for DNA-based testing in at least half of cases (42% for Ion Torrent NGS; 83% for ddPCR depending on thresholds set). A pilot study showed that the yield of DNA can be doubled by the use of the Qiagen circulating free Nucleic Acid kit, increasing the future success rate for DNA-based testing. For reliable results with the ddPCR method, the optimal thresholds need to be determined.<br><br>**Conclusions**<br>DNA-based testing on cyst fluid has additional value for the correct distinction between pancreatic cyst types. In 12.5% of cases an identified mutation was the only indication for a potentially malignant cyst type; in 25% it supported the diagnosis made using other modalities. DdPCR could be used as a pre-screening; if no mutation is found more informative Ion Torrent NGS may be run. |

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| Poster Title | Real-time droplet manipulation and sorting with pneumatic valves. |
| Abstract | Droplet microfluidics is a wonderful enabling technology that promises a plethora of applications in life sciences, namely for lab-on-a-chip and single-cell analysis. Indeed, single-cell technology has attracted huge attention recently with the emergence of new performing sequencing technologies.<br><br>Whereas droplet microfluidics has proved its numerous advantages for single-cell encapsulation in comparison with classical methods, the process still suffers from one main bottleneck: the impossibility to robustly encapsulate precisely one cell in each droplet. This is particularly problematic for applications where co-encapsulation of different single objects in each droplet is required. There is thus a need to actively control the droplets based on their content. Moreover, the real-time observation of the cells in the microfluidic modules would also be highly appreciated by the biologists, as the tools commonly used for single-cell detection and sorting (FACS and flow cytometers) are based only on fluorescence detection.<br><br>We developed a microfluidic device for real-time droplet sorting based on their contents, which consists of a droplet generator with single-cell encapsulation, a detection channel for real-time analysis of droplet contents, and a droplet sorting junction controlled with pneumatic valves. Based on image processing of droplet content, the device is able to sort the droplets with interesting content (e.g. the number of encapsulated particles) with a valve switch up to 250 Hz and 100% droplet switch efficiency. After sorting, further manipulations can be easily performed on droplets.<br><br>Amongst the principal advantages of our system, there is real-time vision: with a large field of view, a large amount of information is available such as the number, intensity or morphology of the encapsulated content. Secondly, the pneumatic valves are easy to fabricate, to integrate in the chip and to control. Moreover, they do not produce any adverse events on viable content, and they are able to immobilize the droplets completely. |

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| Poster Title | Primer set evaluation for quantitative gene expression normalisation in a heterogeneous hMSC sample set |
| Abstract | Human Mesenchymal Stromal Cells (hMSCs) can be isolated from several tissue sources. Being multipotent, they are capable of in vitro differentiation into various cell types such as adipocytes, chondrocytes, and osteoblasts. Human MSCs are further identified by the adherence to plastic in culture and by the expression of three surface molecules (CD105, CD90, and CD73). These criteria seem inefficient for identification since differences between MSCs in terms of their original tissue, isolation method and culture dimension have been indicated by gene expression profiles.<br>Reference genes, which are used for gene expression normalisation and which should be stably expressed regardless of the MSC isolation protocol, culture conditions or extraction method, could provide more reliable markers for hMSC identification. Thus, we cultured hMSCs on both tissue culture plastic (2D) and a variety of scaffolds (3D) before employing several storage and extraction protocols to obtain RNA. This heterogeneous sample set was then used to validate 13 primer sets as candidate reference genes for normalisation of qPCR data. All samples were used to rank the gene stability using BestKeeper, the Delta CT method, Genorm, and Normfinder. We were able to identify the most stably expressed reference genes for gene expression normalisation of hMSC samples. Expression of these reference genes was not influenced by isolation procedure, culture condition, storage, or RNA extraction protocols. |

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| Poster Title | SMARTDIAGNOS – Next generation technology for detection of the pathogens causing sepsis - an EU Horizon 2020 innovation project |
| Abstract | Sepsis is a potentially fatal condition arising when the body's response to an infection, damages its own tissues and organs. Sepsis is one of the biggest health issues in the EU and worldwide due to its high incidence, mortality, and economic cost. Early diagnosis is crucial, as every hour of delay in appropriate antimicrobial (AM) therapy increases mortality by 5-10%. Current methods for detection of the pathogens causing sepsis, including blood culture and different nucleic acid based multiplex amplification technologies, are impaired by the significant time-delay of 1-2 days and low sensitivity of 30-50%. Hence there is an urgent need to develop new diagnostic tools that can provide more accurate and earlier pathogen detection.<br>The proposed SMARTDIAGNOS platforms are aiming to advance sepsis diagnostics by simplifying clinical sample analysis methods and integrating the required numerous steps into a streamlined point-of-care (POC) instrument and a laboratory (LAB) instrument.<br>**Reference**: http://www.smartdiagnos.eu<br>**Acknowledgement**: This research is financially supported by ''SMARTDIAGNOS Next generation sepsis diagnosis technology'' an EU Horizon-2020 project, Grant Agreement No.: 687697. |

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| Poster Title | MSRE-qPCR for multiplexed analysis of DNA methylation can be accurately used for detection and validation of colorectal cancer-specific biomarkers in liquid biopsy samples |
| Abstract | Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide. CRC survival at early detection is 90%, though due to low compliance to and low accuracy of existing screening tools many CRC-s are still discovered in the late stage.<br><br>DNA methylation is a very stable, early and tissue-specific event in cancer development and progression, and detectable in circulating cell-free DNA (cfDNA) derived from blood. Confirming tissue markers in blood however is challenging due to low concentration of circulating tumour-derived DNA (0.1- 1% against non-tumour cfDNA background).<br><br>Methylation-Sensitive Restriction Enzyme (MSRE)-qPCR technology enables detection of <10 copies of targets in highly multiplexed format, making it suitable to detect low amounts of tumour derived DNA in cfDNA. We selected from publicly available tissue methylation data 180 individual CpGs hypermethylated in colorectal cancer and 6 control sites to develop 2x 96-plex assays for analysis with MSRE-qPCR method. Blood was collected from 133 participants (50 CRC, 50 colonoscopy-negative, 11 hyperplastic polyp and 22 non-advanced adenoma patients) attending CRC screening programs and oncology clinics in Spain and USA from 2017-2018. DNA was isolated from the 4ml of plasma available. 1/3 of the isolated cfDNA was not MSRE digested for PCR amplification and functioned as a reference for generation of delta-cts; 2/3 of the cfDNA was used for MSRE digestion to cleave unmethylated DNA and to selectively enrich for the hyper-methylated sites.<br><br>Analysis of control genes and undigested DNA showed high technical reliability of the assays in measuring cfDNA. Statistical analysis on training set (n=30 case, n=40 cont.) identified a 9-methylation marker panel that was further validated on a validation set (n=20 case, n= 43 cont.). 9-marker panel allowed us to achieve detection of stage 0-II colorectal cancer patients with sensitivity of 75% with overall sensitivity as high as 80% at specificity of 90%. Detection was equally efficient for proximal and distal cancers.<br><br>In conclusion MSRE-qPCR technology is a perfect technology for a targeted multiplex approach when only a limited amount of DNA is available. Here it was successfully used for accurate multiplexed analysis of tumour-related methylation markers from plasma samples, thus, contributing into successful development of accurate blood-based tests for early detection of CRC. |

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| Poster Title | Mixing Enhancement in Microdevices Using Synthetic Jets |
| Abstract | As a consequence of the continuous development of microdevices, the improvement of micromixers is required. A great variety of micromixer designs are reported in the literature. However, not all of those microfluidics mixers have reached the commercialisation stage due to difficulties like manufacturing complexity, high cost, complicated control systems and low mixing performance among others. In an attempt to solve the mixing problem at low Reynolds numbers (Re<1), numerous passive and active micromixer designs have been proposed to achieve “good” mixing. Nonetheless, the problem of mixing at low Re still remains despite all the efforts made in the last two decades to design an effective micromixer. In this work, we propose a new active micromixer design based on the synthetic jet principle to improve the mixing performance at Re<1. The micromixer design consists of two liquid streams flowing along a rectangular microchannel and across two lateral synthetic jets. A nondimensional analysis is performed and used to characterise the micromixer design. The results show that a mixing performance of 90% is reached operating the micromixer at L=10.5 (∆pp=3w/4), Str=0.525 (f=6 Hz), Re=0.25 and after t=3 s. The mixing performance is also shown by means of Poincaré maps that confirm the good mixing quality with a well-distribution of particles in the fluid domain. The mixing quality is attributed to the constant stretching and folding of the fluid due to the synthetic jets disturbances. The practical implementation of this micromixer design could have a significant impact on numerous microfluidics processes in the future. |

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| Poster Title | How to transfer a quantitative molecular diagnostic test to multiple qPCR platforms |
| Abstract | **Background**<br>Quantitative gene expression assays are increasingly used for diagnosis and research, but are often restricted to specific instrumentation. We propose a robust technical and statistical framework that enables transferring of established quantitative real-time PCR assays across qPCR platforms without compromising analytical and clinical validity.<br><br>**Methods**<br>The feasibility of our approach was tested on MammaTyper®, an *in vitro* diagnostic assay that quantifies breast cancer biomarkers and dichotomizes results according to cutoff points. CFX96, ABI 7500 Fast, and Mx3000P were chosen as the candidate platforms, whereas the LightCycler 480 II was used as a reference. Two instruments were used per platform, and they were tested initially for equivalence via Bland-Altman and Deming regression analyses. A method comparison approach was adapted to adjust cutoffs for the new systems, and the cross-platform agreement was evaluated. Finally, precision was estimated for each platform.<br><br>**Results**<br>The performance on the candidate devices was highly comparable to the reference platform, with a 7 log quantification range and amplification efficiencies of 97% to 103%. The equivalence tests successfully prequalified instruments, preventing constant and proportional errors and enabling reliable adjustments of cutoffs, which resulted in cross-platform marker and subtype agreements of 91% to 100% and Kappa values between 0.78 and 1.00. A high precision of the quantitative marker assessment was observed on CFX96 and ABI 7500 Fast with standard deviations of 0.14 to 0.24 quantification cycle (Cq).<br><br>**Conclusion**<br>The process described herein proved effective in transferring MammaTyper® to additional qPCR platforms, allowing local pathologies to easily integrate the diagnostic assay in-house, as a technical advance to the current standards of breast cancer marker assessment. Provided that platform-specific adjustments are implemented, the described process can help expand the operability of quantitative diagnostic tests while maintaining assay performance characteristics. |

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| Poster Title | Microfluidic exosome extraction using charged membrane under vacuum pressure |
| Abstract | Liquid biopsy is a novel technique to obtain cancer information from biological fluids non-invasively. Exosome, which is cell-derived nano vesicle, is one of the potent biomarkers for liquid biopsy of cancer since it has been known to carry proteins and nucleic acids related in survival and propagation of cancer cell. In liquid biopsy of exosome, it is important to develop effective purification technique because it is demanding biomarker due to their inherently small size. The gold standard method currently available for exosome purification is ultracentrifugation (UC). However, there has been clinical needs to replace UC to improve several limitations, such as time consuming over 8 hours, high cost, requiring skilled operator and risk of morphological damage. Here, we developed microfluidic exosome extraction chip using positively charged membrane under vacuum. As exosomes have inherently negative charges, a quaternary ammonium membrane with a strong positive surface successfully captured and released exosomes. Use of charged membrane dramatically reduced sample preparation time to less than an hour without ultracentrifuge. Entire workflow (sample binding, washing, and elution) was fully controlled by microfluidic valves and a vacuum pump. Purified exosome was compared to UC in terms of size and concentration using nanoparticle tracking analysis (NTA). The microfluidic exosome extraction showed yield about 7 times higher than UC and a larger vesicle size at 257 nm than 172 nm in UC. In microfluidic electrophoresis assay, our method showed comparative quantity of total RNA to UC under 200 nt size. Exosomal miRNAs (let-7a and miR-142) were quantified by RT-qPCR. It was confirmed that our method successfully purified exosomes preserving miRNAs inside, although higher cycle threshold was observed indicating less miRNA quantity than UC. Therefore, it is expected that the microfluidic chip using charged membrane can be practically utilized to purify exosomes in various clinical applications. |

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| Poster Title | Microfluidic point-of-care test for infectious viruses using isothermal DNA amplification |
| Abstract | Rapid, worldwide spread of epidemic viruses is becoming a serious global problem as the range of travel, speed and number of passengers increases with the development of transportation. When a virus is detected in the field, the best way to avoid further spread is to quickly identify the source of the infection and quickly determine whether to isolate the virus carrier. Therefore, a quick and simple point-of-care test (POCT) must be developed to screen multi-viruses in the initial field. In this study, we introduced a novel method for simultaneous detection of various viruses by DNA hydrogel formation using isothermal amplification method, rolling circle amplification (RCA). Also, the present method was integrated in a microfluidic chip that have bead packing channel which can be blocked due to dumbbell shape of DNA hydrogel formation with positive reaction of virus. The target sensing templates were pre-coated on the surface of agarose bead. Since requiring pressure to initiate stained liquid pass through the blocked channel was affected by the amount of pathogen, it is possible to detect viral infection by measuring the pressure applied in a label-free manner. Large surface area with bead packing effectively reduced complete channel closure time to less than 15 minutes with 0.1 pM of limit of detection. Multiple screening of infectious agents: Dengue fever, MERS, Ebola and Zika was successfully performed using multi-channel integration on a 3D-printed chips. Consequently, it would be expected that our microfluidic technique can facilitate screening for a practical POCT as a proof of concept study. |

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| Poster Title | Microfluidic chip for cell free DNA extraction using immiscible liquid |
| Abstract | Targeted therapy which provide best treatment for each patient has been developed rapidly for cancer management. Cell free DNA (cfDNA), which freely circulating in the blood stream, is one of the potent biomarkers for targeted cancer treatment as well as enables non-invasive tests using blood. cfDNA is present in small quantities of tens of ng / mL, and circulating tumor DNA (ctDNA), which provides important tumor information, is highly rare under 0.1%, so circulating DNA is a demanding biomarker for sample preparation. To purify cfDNA from blood sample, centrifuge-based spin column has been widely used. However, several aspects such as cross contamination, high cost, long process time, and low reproducibility should be further improved for effective sample preparation of cfDNA. Here, we developed centrifugation-free cfDNA extraction method which was named as PIBEX (pressure and immiscibility-based extraction) and the entire sample preparation process was integrated microfluidic chip. The PIBEX method collected about 95% of elution buffer loaded in a silica membrane and effectively replaced centrifugation with vacuum pressure. The microfluidic PIBEX chip was able to extract the amount of cfDNA from a blood plasma comparable to the gold standard method: QIAamp spin column. Furthermore, standard circulating DNA samples including rare mutants rate from 0.1% to 5% were extracted using the PIBEX chip and the ctDNAs were successfully detected in ddPCR analysis. To demonstrate the utility of PIBEX chips in clinical applications, continuous cancer monitoring was performed on PIK3CA H1047R mutations in patients with HER-2 type breast cancer. Severe liver metastasis was observed in MRI images and ctDNA levels in liquid biopsy increased from 12.5% to 57.9%. Therefore, the present study is expected to facilitate an ultimately integrated sample-to-answer system in liquid biopsies. |

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| Poster Title | Development of a fluttering bar-driven inertial micropump imbedded in microfluidic chip |
| Abstract | Lab-on-a-chip (LOC) technology has been widely developed and utilized in a variety of applications, including biomedicine, fine chemicals and precision medicine. Despite this successful miniaturization, microfluidic systems are challenging due to the difficulty of complicated input / output pumping, fluid routing, and fluid tubing. The main causes of these problems are the usage of external pumping system, which are most commonly used for LOCs. Thus, a need for the development of innovative internal microfluidic pumping systems is required. The micropump system must meet the following conditions such as simple, inexpensive, compact, reliable, and easy to integrate into the LOC. Various studies on micropumps for LOCs have been reported including capillary pumps, reciprocating pumps, bubble driven pumps. However, these pumping techniques still do not meet the requirement, because of its limitations such as maximum flow rate, accurate flow rate control, additional check valves and complex external tubing. In this study we developed a fluttering bar-driven inertial micropump, imbedded in microfluidic chip. The pump consists of a rectangular ferromagnetic bar and a unique pie shaped chamber connected with closed-loop microchannel with an inlet and an outlet. The net flow is determined as a function of chamber geometry, location of inlet and outlet, and rotational speed of magnet. Using image analysis, net flow has been shown to be generated by the fluid inertia associated with the reciprocating motion of the ferromagnetic bar. By adjusting the position of the inlet and outlet, one cycle time it takes for the fluid to circulate in microchannel was reduced from 200 sec to 4 seconds. The flow rate of the micropump is 48 - 225 μL/min and is proportional to the rotation speed of the magnet (150 - 3000 rpm). Using a fluttering bar-driven inertial micropump, the microfluidic system not only provides enhanced mixing but also eliminates specific problems associated with external tubing and connection. |

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| Poster Title | Magnetic solid supports handling in droplet microfluidics: application to DNA clean-up and size selection for NGS libraries preparation |
| Abstract | The ability to efficiently purify or extract molecules of interest from a complex matrix is a key component of most bioassays. This is often achieved with **functionalized superparamagnetic** particles as **solid support**. Despite different principles have been presented for the manipulation of such support in droplet microfluidics, a reliable device allowing **enrichment and extraction** of an analyte of interest from a complex mixture with performances comparable with existing lab-scale methods and allowing faster processing times is still missing.<br><br>To answer these needs, we present here the conception, fabrication and characterization of a novel droplet microfluidic approach integrating a **pair of soft magnetic components**, placed adjacently to a microchannel and able to generate a **strong** and **local magnetic force** along the path of the droplet. This device is capable to overcome the interfacial tension of the droplet, retaining the beads contained in it, while the droplet continues traveling in the main channel. The extracted pellet can be either released, upon merging, in a coming droplet or redirected to a secondary channel for further downstream analysis or waste. Combining numerical simulation and experimental characterization, this approach showed remarkable performances, namely the high throughput droplet processing, the **improved extraction/purification rates** compared to existing in-droplet approaches and the important reduction in sample and reagents volumes with respect to conventional lab-scale methods.<br><br>Importantly, we applied this novel technology to the **preparation of nucleic-acids libraries** devoted to **NGS**, focusing on a critical step represented by the DNA size selection process. This protocol involves target DNA fragments capture, based on their precipitation on carboxylic beads in presence of NaCl and PEG, followed by their washing and elution. Our device, integrating both the magnetic separation and the particle extraction and redispersion steps required, allowed for the first time the implementation of this protocol in a continuous droplet microfluidic device. As a matter of fact, we demonstrated **successful clean-up and size selection** stages on a **DNA** ladder sample achieving size distribution and recoveries comparable with conventional methods, but with interesting improvements in reproducibility, robustness and automation.<br><br>Future perspectives include the possibility to integrate the implemented protocols with merging and thermal amplification stages to develop a **sample-in/libraries-out droplet microfluidic platform**, suitable for different NGS libraries preparations. In addition, the robustness and versatility of our concept makes it appropriate for the implementation of further magnetic particle-based **protocols involving purification, rinsing, re-suspension and bio-reactions**. |

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| Poster Title | Optimization of a ddPCR method for detection of fetal microchimerism in healthy mothers |
| Abstract | Maternal-fetal cellular trafficking is the bidirectional passage of cells between mother and fetus during pregnancy. This results in the presence of fetal cells in the maternal circulation, known as fetal microchimerism (FMC). The discovery that these fetal cells can remain present in the mother's body for up to 27 years postpartum further fueled the idea for a biological role for these cells. The biologic role of this bidirectional passage of cells during pregnancy is not known, although it has been implicated in tolerance mechanisms during pregnancy, immune surveillance and tissue repair in cancer.<br>In general, the difficulty in assessing the low FMC numbers (estimates range between 1-1,000 fetal cells/100,000 maternal cells) hampers research in this area. Currently, the method of choice relies on quantifying a Y-chromosome specific region in women who gave birth to sons. However, this limits population studies as mothers of girls cannot be included. Therefore, we suggest using a dedicated panel of whole genome markers to accurately assess fetal microchimerism in mothers. We herewith present the optimization of a protocol relying on the QTrace™ (Jeta Molecular Utrecht, The Netherlands) panel for accurate assessment using digital droplet PCR. The Qtrace™ assays are designed to detect and quantify one individual's genome in the background of another individual or individuals. This method allows the quantification to a level of sensitivity of 1 fetal cell in 1,000,000 maternal cells. Each of the 46 assays is designed to a distinct bi-allelic insertion/deletion (INDEL) or copy number polymorphisms in the human genome. Proof-of-concept data (24 mother/child pairs) show that we are readily able to find unique markers for the baby (ranging from 4 to 15 markers), and *vice versa*, we were also able to identify informative markers for maternal DNA in all samples analysed. The panel of 46 markers contains one located on respectively the X and Y-chromosome, and in our proof-of-principle study the Y-chromosome markers were informative in all samples from new-born baby boys as expected. Furthermore, the X-chromosome marker was informative for 5 out of 12 new-born girls, also according to expectations. 12 of the tested markers seem to be highly polymorphic within the ENVIR*ON*AGE cohort, as these are informative for at least 5 of the 24 samples analysed. |

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| Poster Title | *MLH1* Promoter Hypermethylation: Development and Validation of a Methylation-Sensitive High-Resolution Melting (MS-HRM) Assay for use in a Lynch Syndrome Pre-Screen Pathway |
| Abstract | Lynch Syndrome (LS) is associated with germline mutations in genes encoding the mismatch repair proteins, leading to mismatch repair deficiency (dMMR). dMMR can also arise somatically by promoter hypermethylation-mediated silencing of the *MLH1* gene. In colorectal cancer, there is a strong association between the *BRAF*$^{Val600Glu}$ mutation and *MLH1* promoter hypermethylation, thus analysis of these markers, along with microsatellite instability (MSI), constitute components of the NICE-mandated LS pre-screen pathway. In the absence of a CE-IVD solution, we sought to develop and validate an MS-HRM based, *MLH1* promoter methylation assay for clinical use, using primers and control material from MethylDetect ApS, and bisulphite conversion and HRM-PCR mastermix kits from Qiagen. Paired tumour/normal DNA was extracted from 20 FFPE samples: 12 colorectal, 4 prostate, 2 endometrial, 1 bladder, 1 unknown. Ten samples were MSI-High (MSI-H), 10 MSI-Stable (MSS); 5 were known dMMR, 2 MMR-normal; 4 were known *BRAF*$^{Val600Glu}$, 10 *BRAF*$^{WT}$; 1 from known LS, 3 from suspected LS. 20µL (5ng/µL) DNA was bisulphite converted (bsDNA) and 2-3µL used per HRM reaction. These were run on a Qiagen Rotor-Gene Q with 2-4 technical replicates for 45-50 cycles, with a melt start of 65-69$^{o}$C and finish of 83-95$^{o}$C. The paired-normal sample was used as the unmethylated baseline on a per-sample basis.<br>Repeated optimisation runs showed that 3µL bsDNA input, with 4 technical replicates, x50 cycles, and 69-83$^{o}$C melt were the optimal parameters. All 11 MSS samples scored as unmethylated, as did the 4 known and suspected LS samples. The remaining 6 MSI-H samples (including 3/4 *BRAF*$^{Val600Glu}$) scored as hypermethylated. MethylDetect control samples showed a limit-of-detection of 1% methylation.<br>All validation samples scored as expected, except for one *BRAF*$^{Val600Glu}$ sample that scored unmethylated; this however came from a suspected LS patient and thus warrants further investigation. This assay proved to be rapid, cost-effective, sensitive, and specific. |

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| Poster Title | Dynamic in vitro microfluidic system for standardised osteoimmunological evaluation of implant materials |
| Abstract | In the last few decades, ceramics have entered the field of dental and bone implants and have been subject to extensive implant-related research. Yet there is a variety of suitable ceramic compositions available which remain to be tested for implant research. Furthermore, few studies have investigated the impact of surface conditions such as roughness, surface chemistry, or polarity, and their impact on protein adsorption and cell adhesion and differentiation.<br>At the implant site, the general assumption is that at first blood proteins are being adsorbed by the implant and subsequently immune cells adhere to the surface. Macrophages, as major protagonists in inflammatory responses, are well known to polarize and either promote pro- or anti-inflammatory actions at the wound site. Macrophages signal the surrounding cell types, e.g. other immune cells or mesenchymal stem cells (MSCs), via cytokine release and thereby also have a strong effect on bone forming processes. However, studies focusing on macrophage polarization and their effects to other cells, like MSCs, directly on the biomaterial are still elusive due to varying experimental setups and approaches.<br>With emerging materials and surface functionalizations intended for implant purposes, there is a strong need for a standardized procedure in immunological and osteoimmunological evaluation.<br><br>In this work, we are implementing a modular microfluidic approach that will allow investigation of various materials of interest, co-culturing experiments, and simple and rapid secretome analysis. Our interest is particularly vested in the influence of various solid biomaterials on the polarization of macrophages, and cellular communication between them and osteogenic differentiating MSCs. In a novel approach, we incorporate biomaterials in a circular microfluidic system allowing cytokine signalling of immune cells and MSCs that are spatially separated. Cytokine release and concentrations are being monitored by sampling of circulating supernatant and instant analysis via a homogeneous immunoassay in a separate microfluidic module. |

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| Poster Title | USE OF DIGITAL PCR (dPCR) FOR QUANTIFICATION OF GENETICALLY MODIFIED ORGANISMS (GMO) IN COMPLEX SAMPLES |
| Abstract | Real-time quantitative polymerase chain reaction (qPCR) is widely used for detection and quantification of GMOs. Quantification of GMOs with qPCR method is based on the use of standard calibration curve, prepared from reference materials. Especially for complex samples, quantification can be infeasible due to sensitivity of qPCR to inhibitors from the sample. Digital PCR (dPCR) is a method where the reaction mixture is divided into many individual partitions, enabling absolute quantification without the need for a standard curve. Because of qualitative nature of PCR reaction in partitions, it is less sensitive to partial inhibition that could influence quantification.<br>To overcome the difficulties of GMO quantification by qPCR in complex samples the potential of dPCR was investigated. According to previous studies, direct transfer of qPCR-validated methods to a dPCR system is possible. Thus, we have transferred two validated simplex qPCR assays targeting the soybean species-specific gene and one of the most abundant soybean lines on the world market, commercially known as Roundup Ready® soybean, to dPCR system. These two simplex assays were also merged in duplex dPCR assay to enable more time and cost efficient analysis. The assays were assessed on certified reference materials and complex real-life feed samples using Bio-Rad's QX100™ and QX200™ Droplet Digital PCR Systems and compared to qPCR. Analysed DNA was extracted with two methods to investigate possible influence of DNA extraction on quantification of GMOs in complex samples. |

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| Poster Title | Study of neuronal guidance dynamics in neurodevelopmental disorders models by nano-engineered chips |
| Abstract | In the brain, cells are exposed to both physical and chemical extracellular stimuli determined by the micro/nano-environment within which they exist: these extracellular instructions orchestrate the wiring of the central nervous system. Although the dynamics of neuronal extracellular sensing is emerging as crucial for neuronal connectivity and functionality, little is known about these processes in pathological conditions (e.g. neurodevelopmental disorders). Nano-engineered substrates are emerging as tools for investigating and tailoring the processes that regulate neuronal extracellular sensing. Nano-structured substrates, for example nano-micrograting (GRs), are in fact able to induce specific topographical stimuli to neuronal cells, resembling *in vitro* several features of extracellular matrix (ECM) cues, and consequently to tune neuronal polarity and migration.<br>We here developed and demonstrated nano-engineered chips to study neuronal sensing and migration processes in neurodevelopmental disorders. By exploiting electron beam, nanoimprint and/or replica molding, we engineered thermoplastic (in Cyclic Olefin Copolymer) and elastomeric (in PolyDiMethilSiloxane-PDMS) substrates patterned with GRs (i.e. alternating lines of ridges and grooves) having line-widths between 500 nm and 10 µm. We finally coupled our nano-microstructured substrates with commercial microfluidic chips (IBIDI) with the aim to also expose neurons to chemo-attractant cues. As pathological models, we focused on the unbalanced levels of ubiquitin ligase E3a (UBE3A), which leads to several neurodevelopmental disorders, such as Angelman Syndrome (AS) and 15-duplication autisms (15dup). We tested primary healthy-control, AS and 15dup neurons on nano-engineered chips by applying specific biomimetic environmental physico-chemical stimuli to them and we found guidance deficits in AS neurons.<br>We propose nano-engineered platforms as advanced devices for investigating neuronal guidance dynamics *in vitro* at molecular level, as in an enriched environment able to resemble closely the *in vivo* conditions. This work is supported by MSCA-IF-2017 grant Neuroguide (795948). |

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| Poster Title | High-throughput, full-length, single-cell RNA sequencing |
| Abstract | Since the emergence of next-generation sequencing (NGS), the importance and demand for single-cell analyses have risen rapidly. Single-cell RNA-seq can be used to generate data for analysis of differential gene expression, alternative splicing, gene fusions, and so on, allowing for cell profiling in detail. As a result, this technology has been gaining prominence not only in basic research fields, but also in the clinical space. Therefore, automation systems that can process many single cells regardless of cell type or size, and that provide high-quality data with high sensitivity, reproducibility, and full-length sequence information are desired. Our method presented here for full-length RNA-sequencing the SMARTer™ ICELL8® automation system meets the demands described above.<br>Our SMARTer ICELL8 system is an automated platform specialized for single-cell analysis. Unlike other automation systems, the SMARTer ICELL8 system utilizes a simple dilution method with a large aperture dispenser for single-cell isolation, enabling users to characterize many types of cells in a small amount of time. In addition, the SMARTer ICELL8 system provides the capacity to image and select cells for downstream analysis on a well-by-well basis, such that >1700 single cells can be analyzed at once.<br>Our method for full-length RNA-sequencing the SMARTer ICELL8 system will enable users to perform all reactions required for Illumina® NGS library preparation in the ICELL8 chip, including cDNA synthesis and PCR amplification, so that the output from the chip is a sequencing-ready library. The most important feature of our approach is that the output library contains full-length sequence information as opposed to only capturing RNA 3' ends (data typically used for differential gene expression analysis). Along with incorporating SMART® template-switching technology to ensure production of high-quality cDNA, our method for full-length single-cell RNA-seq with the SMARTer ICELL8 system enables various analyses to be performed with high sensitivity and reproducibility. |

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| Poster Title | Mimicking the *in vivo* environment of the oviduct in microfluidic channels for the *in vitro* culture of mammalian embryos using hydrogels and 3D printing technology |
| Abstract | **INTRODUCTION**<br>When fertilization occurs naturally, the zygote is surrounded by a constantly changing dynamic environment, which supports the embryonic development. 3D printing technology can be used to mimic the structures of the *in vivo* oviduct, which can give interesting insights into mechanisms stimulating early embryonic development. We aim to create a more *in vivo* like habitat to explore the different effects of physical and mechanical origins on *in vitro* embryo development. Here, we use 3D printing technology for printing scaffolds and evaluate their fitness for creating microfluidic structures in polydimethylsiloxane.<br>**MATERIALS AND METHODS**<br>We compared the suitability of four scaffold materials for simplified structure shaping based on: polyvinyl alcohol (PVA), polylactic acid (PLA), polyethylene terephthalate (PET) and acrylonitrile butadiene styrene (ABS). For PDMS, we need a temperature of preferably 60-80 °C to get quicker curing. Therefore we looked at characteristics of the scaffold materials such as melting temperature, ease of handling, and print quality in an Ultimaker 3D Printer.<br>**RESULTS AND DISCUSSION**<br>ABS is the preferred material for scaffold design, and as a result channel fabrication in PDMS, for its ability to withstand higher temperatures (melting temperature 105°C) and a very good print quality. However, despite the lower melting temperature of PLA (60°C), it still represents a very useful scaffold because it offers the best print quality. Both are easy to handle. We will use both types of scaffolds for forging microfluidic structures in a PDMS mould, which is less elastic than the oviduct surface. In order to more closely mimic the natural environment of the oviduct, our research will be expanded by using hydrogel materials on top of the PDMS, which will supply a more soft bedding for *in vitro* bovine embryo culture.<br>**CONCLUSIONS**<br>We have identified two materials, PLA and ABS which can be used to create microfluidic channels in PDMS for mimicking the oviduct during *in vitro* embryo culture. |

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| Poster Title | Unravelling long genomic DNA molecules under pressure driven flow |
| Abstract | Microfluidic channels are investigated with a view to unravel and deliver long genomic DNA strands (>20μm) into channels for subsequent optical interrogation. Based upon our experimental studies, our results show that curved microchannels deliver more unravelled and stretched DNA strands than straight channels. Numerical studies of the dynamics of single DNA molecules under pressure-driven flow support the experimental findings and provide an insight into the subtle interaction of diffusion, microfluidic shear forces, and elastic molecular forces. The outcomes have yielded a microfluidic technology for unravelling of DNA for 'threading' DNA into optical interrogation channels. |