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MICROFLUIDICS PLATFORMS TOWARDS  
SAMPLE PREPARATION, NUCLEIC ACID  
IDENTIFICATION AND NEXT GENERATION  
SEQUENCING FOR ON-SITE APPLICATIONS

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There is an increasing need for on-site applications of DNA-based methods, both, for inspection services, as well as for companies that wish to monitor their supply chain. The capability to apply DNA-based methods in the field, or at 'point-of-use', is critical for the practical application of DNA-based methods where centralised laboratories may not be able to meet the demand of prompt answers for early intervention, e.g. in logistic chains with short turn-around times. The application fields addressed by DECATHLON are food analysis with specific emphasis on pathogen detection for food safety, GMO identification and customs issues. In recent years much progress has been made in the development of different types of on-site devices that can accommodate DNA-based methods. Within the DECATHLON project two primary approaches for on-site use of DNA-based methods are developed in parallel. The first route is focussed on sample preparation and nucleic acid identification using a centrifugal microfluidic platform. The second focus is the development of a novel sensor, based upon graphene nano-gaps that will be used to detect multiplex targets in processed samples. The centrifugal 'lab-on-a-disc' platform is of increasing interest in the microfluidics community particularly for low-cost, rugged point-of-use applications such as medical diagnostics and environmental monitoring. This paper will present results taken from a 'DNA-to-Answer' cartridge. This platform, for the first time, will demonstrate the spatially multiplexed detection of a DNA sample by discretising a sample into 12 individual wells. Combined, using valving, with LAMP reagents and primers, this disc is capable of addressing 12 different gene targets; towards identification of Shiga Toxin producing E. Coli (STEC) serotypes. This approach, compared with fluorescent multiplexing, simplifies the reagent assays and the required fluorescent instrumentation; thus contributing to the ruggedness of the platform. Nanogap detectors are very promising for genomic screening, in particular DNA sequencing. Graphene nanogaps (single atom thick), can – in principle – achieve single nucleotide resolution (only one nucleotide inside the gap vs 100 nucleotides inside the conventional ~30 nm thick solid state pores). Nanogaps can be patterned in a freestanding monolayer of graphene with high precision using TEM lithography. However the free standing membrane is made unstable and brittle in the process of patterning electrodes (which is necessary for electrical detection). Membrane tension can induce tearing of the graphene upon liquid incubation and therefore result in subsequent folding and rupturing of the freestanding structures. This section will present the investigation of alternative fabrication protocols. This represents the first steps towards integrating a graphene based sensor onto a point-of-use food analysis platform for next generation sequencing.

**Keywords:** on-site application, centrifugal microfluidics, sample preparation, graphene, next generation sequencing

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