Disc-shaped Point-of-Care Platform for Infectious Disease Diagnosis

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Abstract:

Malaria is one of the most prevalent infectious diseases in tropical areas of the world. According to the World Health Organisation (WHO) 2010 records, there were 174 million malaria cases reported, 80% of which were in Africa, leading to 600 000 deaths. The patients present themselves with acute fever as the only clinical symptom, which complicates the diagnosis because in Africa and other tropical regions acute fever is the clinical indication of several other diseases, such as dengue and chikungunya (viral infections), as well as typho and pneumonia (bacterial infections). In fact, it is reported that approximately 30-40% suffering from fever are given anti-malarial drugs while in reality they suffer from another disease. Furthermore, there is an increased risk for spread of the aforementioned diseases to developed countries due to globalization, climate change, immigration and returning travelers from malaria endemic regions, which raises the need for diagnostic efficiency.

Existing diagnostic methods are mostly the microscopy smear tests (specially for malaria) and Rapid Diagnostic Tests-RDTs based on immunodetection. These tests, however, have often been disputed for their reliability and accuracy. Despite their low cost, the fact that they are specific to only one single disease marker often necessitates the use of more than one RDTs, thus, increasing the overall cost. The DiscoGnosis project develops a point-of-care diagnostic platform, aiming to detect several diseases with similar clinical symptoms in a rapid, specific and automated way. A number of novel technological approaches are implemented towards this scope, described below along with the advantages they offer:

Assays: Apart from malaria, the diagnostic panel includes dengue, chikungunya, typho and pneumonia. Such diverse panel requires that the diagnosis is based on highly specific molecular marker detection. Depending on the time elapsed from the onset of the disease, different molecular identification is necessary. Therefore, both, nucleic acid targets and protein markers are monitored in order to span a large diagnostic window. The implementation of LAMP technology for nucleic acid amplification and detection offers high specificity due to its multi-primer nature. The low energy demand of LAMP due to its isothermal profile is particularly advantageous compared to PCR in point-of-care diagnostics.

Analytics: Magnetic beads are used as carriers to form a bead array that will be probed by an integrated camera. Fluorescent nanoparticles (Quantum Dots – QDots) at various colors/wavelengths will be conjugated on the magnetic beads, acting as the identification and

quantitation agents. The detection optics are based on single-wavelength UV excitation of the QDots and subsequent fluorescent imaging with an integrated camera. This approach enables multiplexity and allows several targets to be detected in a single reaction chamber with one “shot”.

Automation: Assay and analytics components are converged in a disc-shaped microfluidic platform, which uses centrifugal and capillary forces to manipulate fluids. The advantage of this approach is the lack of external pumping instrumentation and interface with tubes since the centrifugal microfluidics are essentially self-driven. The sample handling is based on the concept of unit operations, i.e., individual microfluidic structures each serving a discrete fluidic function and altogether interfaced appropriately on the disc. Within this concept, all operations are carried out on disc, for example plasma isolation for protein analysis, cell lysis and nucleic acid extraction and purification for genetic analysis. Towards the full automation, pre-storage of reagents is a core objective: enzymes, primers, amplification components are dry-stored on the disc and re-hydrated; liquid buffers are pre-stored in special “packets” called “stickpacks”, which open controllably at will (based on centrifugal burst pressure) to release the buffers.

Production: The fabrication of disposable cartridges is based on micro-thermoforming, i.e., the modification of the standard thermoforming technology (used for producing blister packages for food and/or pharmaceutical purposes) into microstructure-compatible production process. The typical steps for fabricating the final kits are: thermoforming; filling with dry/liquid reagents; sealing; cutting and packaging. The polymer-foil nature of the microfluidic cartridge allows adaptability to low-cost scalable production in large batches.

Validation: The developed system will be validated in clinical settings at the areas of need, in local hospitals in Africa via partners’ established contacts. The development steps take into account local particularities such as high temperature and humidity, frequent electricity cuts etc. In parallel, the results will be compared with the “gold standard” methods used for the examined diseases to assess the reliability of the system. Finally, the clinical interpretation of the acquired multi-marker diagnostic data will be done by the clinical experts of the consortium.

The project runs since November 2012 for three years. In light of commercialization, most experimental processes (e.g., fabrication, assays, micro/nanoparticle production) will be quality-control approved and the final device will be CE-certified. Further information about the project is available at www.discognosis.eu.

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