

From sample-to-answer: Lab-on-chip platform enabling diagnosis of coeliac disease

This work was carried out in context of the FP7-2007-ICT-1-216031 project CD-Medics. Runtime from February 2008 to July 2012

Introduction

Coeliac disease (CD) is a disorder in which genetically predisposed individuals develop small intestinal inflammations upon exposure to dietary gluten. After successful diagnosis and withdrawal of gluten from the diet, the process can be reversed leading to the disappearance of the small bowel abnormalities. Although medical studies showed that CD should affect 0.5% to 1% of the European population (i.e. 4-7 million patients), it remains severely underdiagnosed and estimated 85% of the cases are unrecognized which is – amongst other reasons – due to the requirement of considering multiple parameters during diagnosis.

Measurements of serum IgG and IgA anti-gliadin antibodies, followed by intestinal biopsy in the case of positive response, were previously used to detect CD. However, the serum test can give positive results on non-coeliac individuals with conditions such as gastroenteritis, inflammatory bowel disease

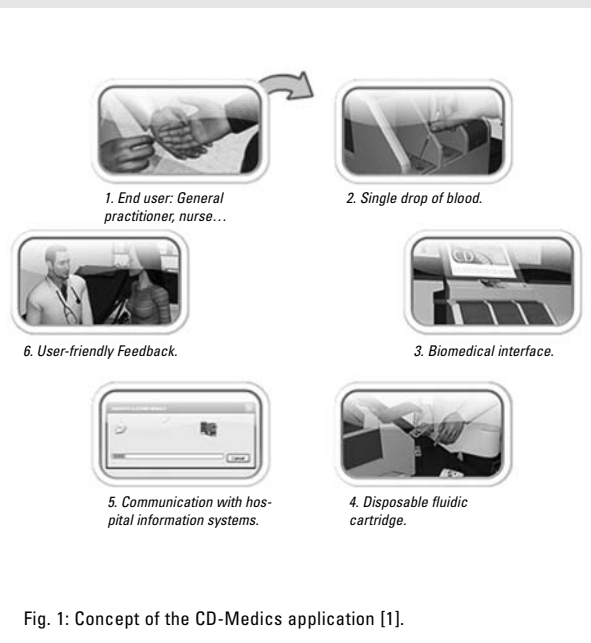


Fig. 1: Concept of the CD-Medics application [1].

and cow's milk protein intolerance. In parallel, a strong association between the presence of certain human leukocyte antigens (HLA) in the patient and CD could be shown. Despite that finding, HLA typing to ascertain patient susceptibility is rarely carried out due to its expense.

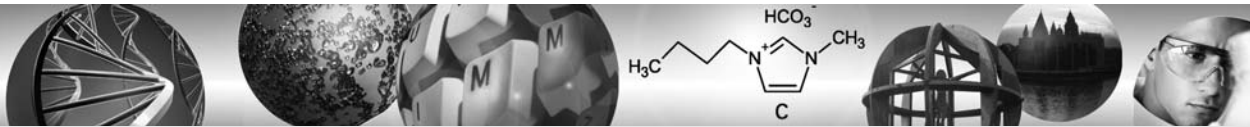
Main objective of CD-Medics is to develop a low-cost application enabling fast and reliable diagnosis of CD, whilst requiring minute amounts of blood from the patient (micro-litre range). Taking advantage of microfluidic technology, a Lab-on-Chip system is being developed that offers the possibility to perform HLA typing and serum analysis at the point of care (cf. Fig. 1).

The final, integrated CD-Medics instrument will act as a device in the clinical-analytical laboratory or in the doctor's office, aiding the physician to arrive at a clear diagnosis by performing complex multi-parameter analysis.

CD-Medics Tasks and Competences of IMM

In this European joint project [2] involving more than 20 academic and industrial partners, IMM assumed responsibility for specifications and design review of the overall system as well as for the development of microfluidic disposable cartridges being the platform for all bio-analytical processes required in the multi-parametric approach for CD diagnostics. In cooperation with partners (listed on the next page of this flyer) designs for both, HLA typing and serology assay, were devised, integrating all specific sample preparation steps and analyte detection arrays. Both cartridges will be equipped with an identical interface to the instrument allowing for operation in the same universal slot. The major chal-

lenge of achieving layouts consistent to the interface was approached by integration of standard as well as innovative, compact Lab-on-chip solutions for assay specific sample preparation methods – e.g. separation of blood cells and plasma, cell lysis, DNA isolation and amplification, electrochemical detection. Notably, a novel PCR-on-chip module based on the moving-plug concept [3, 4] has been designed and integrated in to the HLA typing cartridge. Most prominent advantages of the module are the small footprint and high temperature ramping rates allowing for 30 PCR cycles being completed within 6 min.



Design of disposable microfluidic cartridges and results obtained

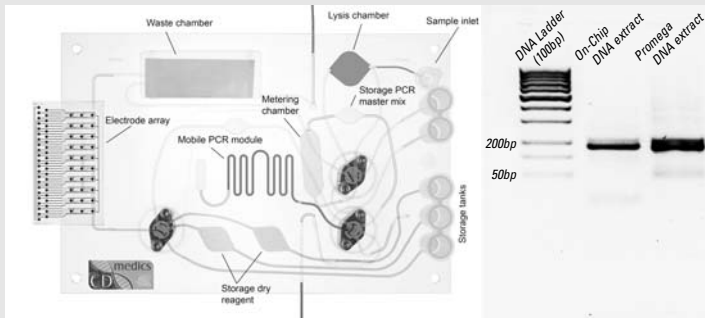


Fig. 2: Layout of the cartridge for HLA typing [5, 6].

In the concept of the overall device all elements required to control timing, local temperature, flow of reagents, data acquisition and interface to the Hospital Information System (HIS) and Electronic Patient Records (EPR) are provided by the universal actuation and read-out instrument. The disposable polymer cartridges (HLA and serology), however, hold all microfluidic modules coming into contact with the patient blood.

HLA-typing on the cartridge depicted in Fig. 2 comprises the following steps: a) insertion of the cartridge into the instrument slot, b) introduction of a drop of patient blood in the sample inlet, c) lysis of

Summary

Within the framework of the EC project CD-MEDICS [7] a minimal-invasive point of care application based on Lab-on-chip technology allowing for reliable diagnosis and monitoring of coeliac disease has been developed. The instrument will, by using a fingerprick drop of blood be able to perform both, HLA typing to determine

References

- [1] www.cdmedics.eu
- [2] http://cordis.europa.eu/fetch?CALLER=PROJ_ICT&ACTION=D&CAT=PROJ&RCN=85545
- [3] J.-Y. Cheng, C.-J. Hsieh, Y.-C. Chuang and J.-R. Hsieh, *Analyst*, 2005, 130, 931-940
- [4] G. Münchow, D. Dadic, F. Doffing, S. Hardt and K. S. Drese, *Exp. Rev. Mol. Diagn.*, 2005, 5, 613-620

Partners for Cartridge and Instrument

- Universitat Rovira i Virgili, Tarragona, Spain
- microfluidic ChipShop GmbH, Jena, Germany
- inno-Train, Kronberg, Germany
- Eurospital, Trieste, Italy

Fig. 3: Gel chromatography of amplified DNA extract; left: DNA ladder; middle: on-chip extracted DNA from whole blood sample; right: commercially available DNA extract [5].

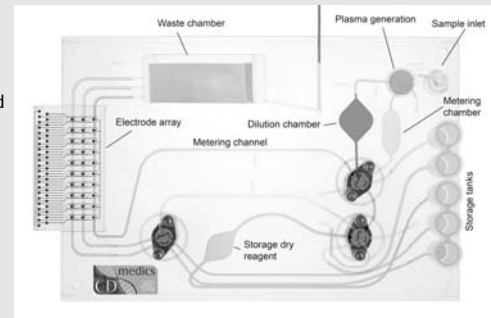


Fig. 4: Layout of the cartridge for serology [5, 6].

collected white blood cells, d) magnetic bead assisted purification of DNA, e) amplification within the PCR-module, f) removal of excess primers, and e) detection based on electrochemical sensing. In Fig. 3 it is demonstrated that the quality of the DNA sample extracted on-chip is similar to genomic DNA material purified by conventional methods.

In the serology microsystem, the purified blood plasma (Fig. 4) is distributed over the sample area of the electrode array allowing the measurement of the disease indicating markers. Parallel measurement of a reference serum allows for quantitative statements.

whether the individual has a predisposition for the disease and a serology test to determine whether the disease is expressed or not. The serology test will also be used for ongoing monitoring of patients undergoing a gluten free diet.

- [5] M. Jung, J. Höth, J. Erwes, D. Latta, X. Strobach, T.E. Hansen-Hagge, R. Klemm, C. Gärtner, T.M. Demiris, C. O'Sullivan, M. Ritz-Lehnert, K.S. Drese, *Proc. SPIE*, 2011, Vol. 79290, 79290D/1-5
- [6] R. Klemm, N. Hlawatsch, C. Gärtner, M. Jung, J. Höth, C. O'Sullivan, H. Becker, *Proc. SPIE*, 2011, Vol. 79290, 79290D/1-5
- [7] http://www.imm-mainz.de/index.php?id=cd_medics