

# Lab-on-Chip Solution for the Detection and Monitoring of Coeliac Disease

This project operates in the context of the FP7-2007-ICT-1-216031 project CD-Medics. It is partially funded by the European Commission 7th framework programme.

## 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 under-diagnosed and estimated 85% of the cases are unrecognized which is – amongst other reasons – due to the lack of awareness of the disease and to the multiple parameters that have to be taken into consideration 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 gastro-

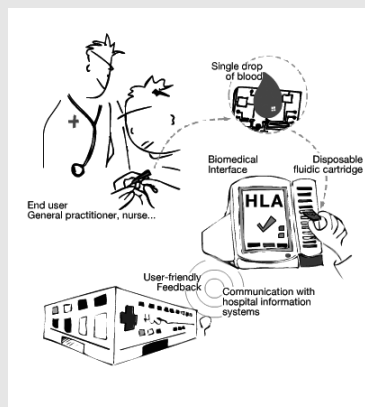


Figure 1: General concept scheme of CD-Medics ([www.cdmedics.eu](http://www.cdmedics.eu)).

enteritis, inflammatory bowel disease 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 to CD is rarely carried out due to its expense.

The key target of CD-Medics is to enable the fast and reliable diagnosis of CD, requiring only minute amount of blood sample (microlitre range) and at low costs. Taking advantage of microfluidic technology, a Lab-on-Chip system is being developed, offering the possibility to perform HLA-typing and serum analysis at the Point-of-Care (Figure 1).

The final, integrated CD-Medics instrument will act as a device in the clinical-analytical laboratory or in the rooms of a general practitioner, performing complex multi-parameter analysis and supplying the physician with a clear diagnosis.

## Competences

In this European joint project involving more than 20 academic and industrial partners, the IMM took the lead of the overall concept design as well as the development of the microfluidic chips being the platform for all bio-medical processes for multiparametric CD diagnosis. In this regard the primary task is to conceive microfluidic cartridges for both, HLA-typing and serology assay. They comprise all the specific sample preparation steps and analyte detection arrays, but share the same interfacial connection ports. In this way, either chip will be run in the

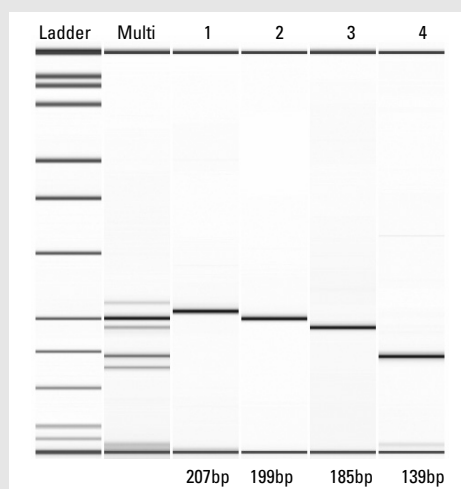
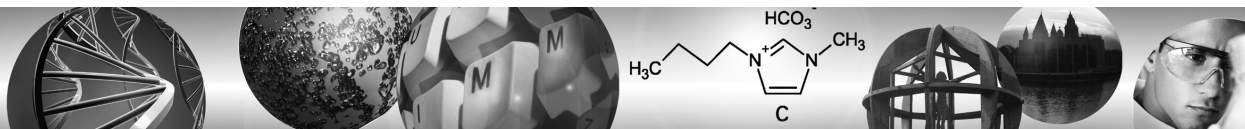


Figure 2: Pattern of PCR amplified fragments after electrophoresis and analysis on a Bioanalyzer(TM) (Agilent). Amplification experiments were performed with isolated fragments as well as multiplex.

same slot of the device. The challenges hereby rely in the integration of assay specific sample preparation methods – e.g. separation of blood cells and plasma, cell lysis, DNA isolation and amplification, detection – in such a way that the interfaces to the instrument remain identical. For this purpose, a new PCR-on-chip module has been designed, taking less space than conventional system and at the same time increasing dramatically the speed of the reaction (complete PCR below 15 minutes). Preliminary PCR results obtained with the chip are presented in Figure 2.



## Setup

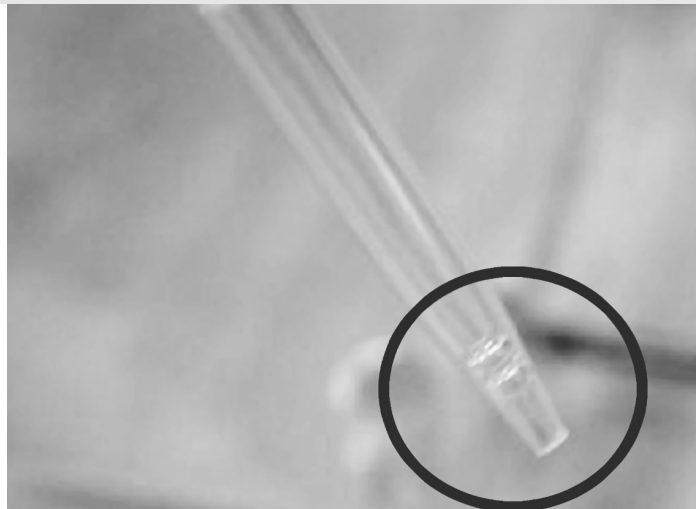
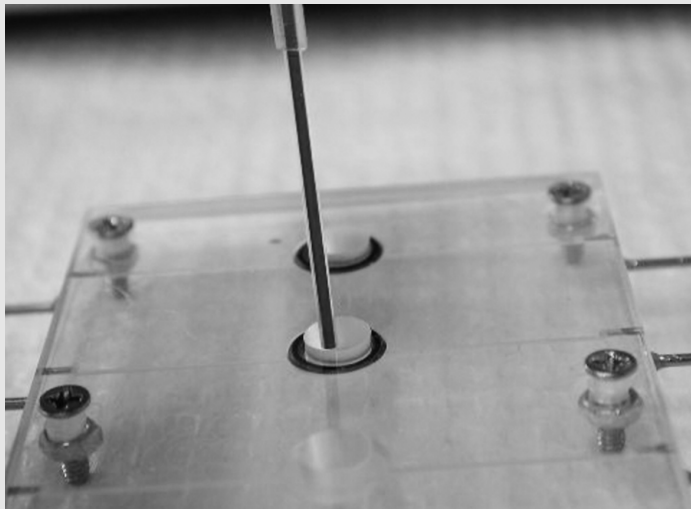


Figure 3: Blood plasma extraction from whole blood via membrane filtration.

The system relies on two different microfluidic cartridges for both, HLA-typing and serology assay that display the same interfacial connection to the instrument thereby enabling a permutable use of both sorts of chips in any slot of the instrument. The tests are performed on disposable microsystems which contain all of the assays, reagents, and sensors needed. To run a test, the chips will be inserted into the instrument and a drop of blood added. The device will control all required parameters such as timing and local temperature, flow of reagents, collection of analysis data, and communication of these data to the Hospital Information System (HIS) and Electronic Patient Records (EPR).

For the HLA-typing cartridge, whole blood is introduced into the system, the collected white blood cells are lysed, and the purified DNA is amplified in the on-chip PCR-module. Excess of free primer is fished out and the amplicates are loaded on the re-hydrated electrodes. Secondary DNA label and the mixture of substrate and mediator are then dissolved and distributed over the electrodes, thereby finally allowing an analysis of patient's HLA-configuration.

In the serology microsystem, the purified blood plasma (Figure 3) 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.

## Summary

The objective of the CD-Medics project is to develop a minimal-invasive Point-of-Care prototype instrument for the diagnosis and monitoring of coeliac disease. The instrument will, by using a finger-prick drop of blood, be able to perform HLA testing to determine

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.

[www.cdmedics.eu](http://www.cdmedics.eu)