Contract no. 216031

CDMEDICS

Celiac Disease Management Monitoring and Diagnosis using Biosensors and an Integrated Chip System

INSTRUMENT: Large Collaborative Project

PRIORITY: FP7-2007-ICT-1

Project Periodic Report

(Publishable Summary)

Annex 1 dated: 15 November 2011

Periodic report: **1st** □ **2nd** □ **3rd** □ **4th** ■

Period covered: **1 February 2011 to 31 July 2012**

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1. Publishable Summary

CD-MEDICS is a 54 month project co-funded by the European Commission researching Point of Care diagnostics for Celiac Disease (CD). CD is a disorder that affects 1% of the European population. It is an auto-immune condition triggered by eating gluten which results in inflammation of the small intestine and malabsorption of nutrients. Symptoms can include anaemia, hair loss, joint pain, behavioural problems and severe fatigue as well as gastric symptoms (although not in every case).

If untreated the condition can lead to infertility, osteoporosis and bowel cancer as well as many other conditions. Diagnosis can be difficult which can result in inappropriate treatments, hospitalisation, and the use of unnecessary drugs. It is not unusual for it to take many years for an accurate diagnosis. Furthermore non-diagnosed and non-treated CD can increase the likelihood of developing other conditions such as diabetes mellitus, auto-immune thyroiditis and many others. Current diagnostic methods require blood tests which look for the antibodies associated with celiac disease followed by a biopsy looking for gut damage typical of the condition.

CD-MEDICS aims to develop a point-of-care technology platform for diagnosing and monitoring of CD. Using a fingerprick blood sample, it will be capable of detecting celiac genes (HLA DQ2/DQ8), if present in an individual, thus indicating a potential predisposition to the disease. It will also detect if there are relevant autoantibodies in the blood indicating that the disease is present and that gluten is in the diet. This approach can thus remove the need for a bowel biopsy. The tests will take place in small disposable microfluidic cartridges which will contain assays, reagents, sensors and fluidic control mechanisms. This will be inserted into, and controlled by an instrument, with a footprint similar to a laptop computer, that will analyse the data from the sensors and provide the information to the clinician.

The system will also have communication capabilities for direct interfacing with hospital information systems allowing doctors to access patient information and history quickly and easily. The instrument will be a low-cost non-invasive intelligent diagnosis system that can be present at point of care such as a doctors´ surgery, which will bring benefits to patients and healthcare professionals.

The work is split into a number of workpackages covering the overall design, assays for antibody and genetic testing, microfluidics, sensors, instrumentation and communications, as well as workpackages focused on informing the general public about celiac disease and developing continuous professional development courses and modules for healthcare professionals, advising them on the symptoms, diagnostic and therapeutic pathways for celiac disease.

The work within the project is also augmented by investigation of the commercial opportunities for the various products under development and the intellectual property generated.

Considerable effort has been put into defining the specifications of the required assays, sensors, microsystem, instrument and communication capabilities from key stakeholders' perspectives. This has laid the ground work for the project and enabled the development to be focused and coherent. The significant findings of this process are:

1) Test times should not be significantly longer than 15 minutes.
2) Separate microfluidic cartridges for HLA and Antibodies as the HLA test is only performed once.
3) Selling prices for both, disposable chip and instrument, need to be aligned to the medical market and the many procurement differences between healthcare providers.

This has resulted in a set of specifications, and consequential requirements for assay development, biosensor arrays, microfluidics, instrumentation, and communications. Based on this, the project has been developing and testing of a plethora of assay formats, electrode array requirements, microfluidic components, as well as potential instrumentation designs. The communication protocols have also been evaluated on and off the actual envisaged instrument.

Within the assay development part of the project several strategies were developed and tested in order to make a standard for anti-tTG antibody. It was found that the best method was based on tTG immobilised on magnetic beads. A batch of anti-tTG antibody was then isolated from serum. However a large amount of human serum albumin was also present in the sample; therefore a second purification was carried out using an albumin-depletion kit and column chromatography. The activity of isolated standard was tested by ELISA. This allowed the quantification of the amount of anti-tTG equivalent to the arbitrary unit used in the commercial kit. This result will be extremely valuable to the new diagnostic criteria recently released by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN).

Additional work on the Development & Exploitation of an HLA-RA association assay has taken place. This work is important to show, that the consortium is able to extend the DNA assay developed to detect the CD associated alleles to other targets. Therefore INNO designed and tested new PCR-SSP primers to detect the alleles, who contribute to susceptibility and rate of progression of joint destruction in rheumatoid arthritis (RA): DRB1*04 (*04:01, …), DRB1*01 (*01:01, *01:02), DRB1*10:01 and DRB1*14:02.). These alleles can be found in up to 80–90% of caucasoid RA patients. After establishing single- and multiplex assays with standard primers, the special modified primer variants were successfully tested in singleplex and multiplex assays.

Within the sensor development work in this period stability studies and validation of biosensors were the main tasks achieved.
The stability over time of both approaches (supramolecular and antigen or DNA covalent linked via self-assembled monolayers) were tested at 4ºC and 37ºC (as accelerated study).

The comparison of stability between surfaces stored with and without a stabilising agent (StabiltCoat® Plus, from Surmodics) was also carried out.
The surface chemistry based on thiolated DNA showed a higher stability over time than that based on supramolecular interactions. From accelerated results was possible to estimate that the thiolated DNA biosensor can be stored for more than 2 years at 4ºC.
In the case of the serology biosensors, different antigen-coated surfaces were tested: digested gliadin peptide (DGP), tissue transglutaminase (tTG) and IgA antibodies (IgA). Similar to the DNA case, covalent thiol-gold linked surface showed higher stability than that observed by supramolecular approach. From accelerated studies these surfaces demonstrated long term stability at 4ºC with StabilCoat.
From the results of the stability studies it was thus decided to use the self-assembled approach for validation of biosensors using real serum samples. 17 real samples from patients (diluted 1:100) were analysed to detect anti-DGP and anti-tTG. In addition 11 IgA serum samples from IgA deficient patients were tested. The results obtained correlated in excellent way with those obtained using a standard sandwich ELISA assay.
Both intra (5 times same sample in the same array) and inter-array (5 samples in two different arrays) assay validation were carried out with excellent correlation. Inter-personal and inter-laboratory validations were also accomplished at URV and MFCS with excellent correlation. In the case of HLA-DNA biosensor, the SSP approach was used. The gold electrode surface was modified by three capture probes: 3b, 4y, 5z and HGH as positive control for PCR, and multiplexing detection was achieved. 23 PCR samples, diluted 1:5 in Tris buffer containing 1 M of NaCl, were tested. Excellent correlation was obtained not only in the validation at URV, but also in the inter-laboratory validation accomplished at URV and MFCS facilities.
The final validation step in a hospital environment is currently being completed. For this, the instrument and chips have been moved to a real application scenario at the Department of Pediatrics of University Medical Centre Maribor.
In addition, to demonstrate the versatility of the biosensor platform developed in this project, rheumatoid arthritis (RA) was detected in three PCR products provided by Inno-Train. The PCR products were modified with the same tail than those used in celiac disease detection. The detection of RA using other real samples is under evaluation.

As recommended in the period 3 Technical Review report, UNEW re-allocated effort to the development of a stand-alone multi-electrode array with a custom built multi-channel Frequency Response Analyser (FRA) to interrogate the array. This included work on hardware, software, microfluidics and sample measurement protocols for clinical evaluation studies to be performed during the period February 1st 2012 to July 31st 2012 under workpackage 7, Task 7.4, Clinical evaluation of developed microsystems. As an outcome, UNEW successfully developed the stand-alone sensor array system for impedance detection using electrode arrays and a multi-channel FRA to produce a laboratory-based system.

The fabrication of the integrated microsystems design was modified and adapted for an injection molding fabrication processes. The injection molding tools and inserts, containing the microstructures were fabricated for the HLA typing, serology and combined serology microsystem. For all three microsystems the molding process was established and

approximately 1000 microsystems of each were realized. The figure below shows the injection moulded HLA and serology microsystems.

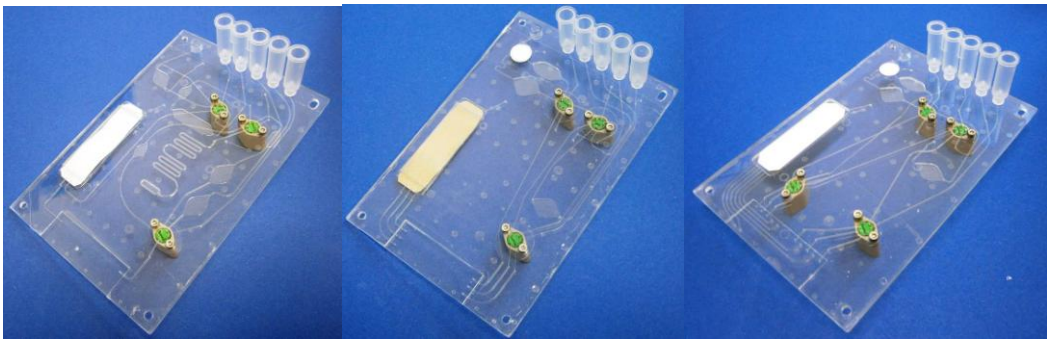

Figure 1 Injection moulded HLA, single serology and dual serology microsystems

In terms of validation of fluidic and functional modules the work of WP4 was focused on the core modules of the integrated microsystem, like turning valves and dry and liquid storage on the technical side as well as DNA extraction and PCR on the application side. For all these modules studies of reliability, inter and intra reproducibility, and performance. This work was done in close cooperation with WP7.
A lot of work was undertaken for the evaluation of the fluidic and biological process protocol evaluation in this period. Thereby the goal was to realize the foreseen fluidic work flow planned for the integrated HLA typing and serology microsystems. The system of microsystem and instrument was adjusted for optical and fluidic interfaces, files for the optical alarm point were generated and modified according to the process behaviours and actuation routines were tested and evaluated.

The instrument prototype has undergone an additional iteration to produce a more compact prototype. In addition significant industrial design took place to produce a prototype instrument much closer to a product representation. The 3 prototypes are shown below.

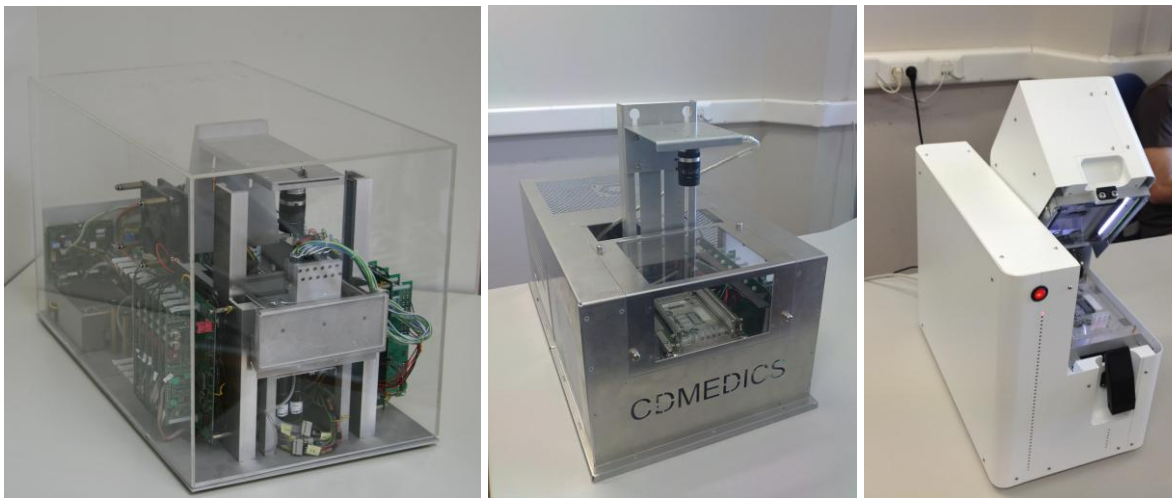

Figure 2 The three generations of instrument prototypes in CDMEDICS.

The 3rd generation instrument prototype features a series of characteristics that were not present in previous versions, such as an efficient ventilation, easy accessibility of all serviceable parts, optimized cabling, reduced tubing (drastically reducing the dead volume problem of previous prototype generations), mechanisms for the avoidance of instrument

contamination, mechanical adaptability of the slot to different types of microsystems (both thickness of the microsystem, as well as the height of microsystem-attached depots are fully adjustable), novel lighting sources and waveguides, etc.

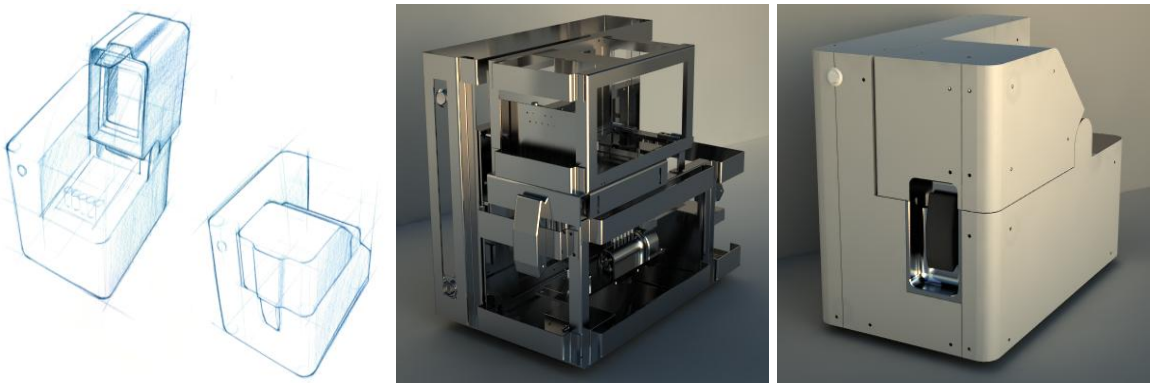

Figure 3 The roadmap to the new instrument design: on the farthest left the initial conceptual drawings, in the middle the new compact interior of the instrument and on the right the complete redesigned instrument prototype.

In addition the fluidic motion monitoring and control has been significantly improved during the period featuring a new camera setup and enhanced software. The key advantages of this approach are reduced cost and flexibility provided to microsystems design. The figure below shows a snap shot of the fluidic control process.

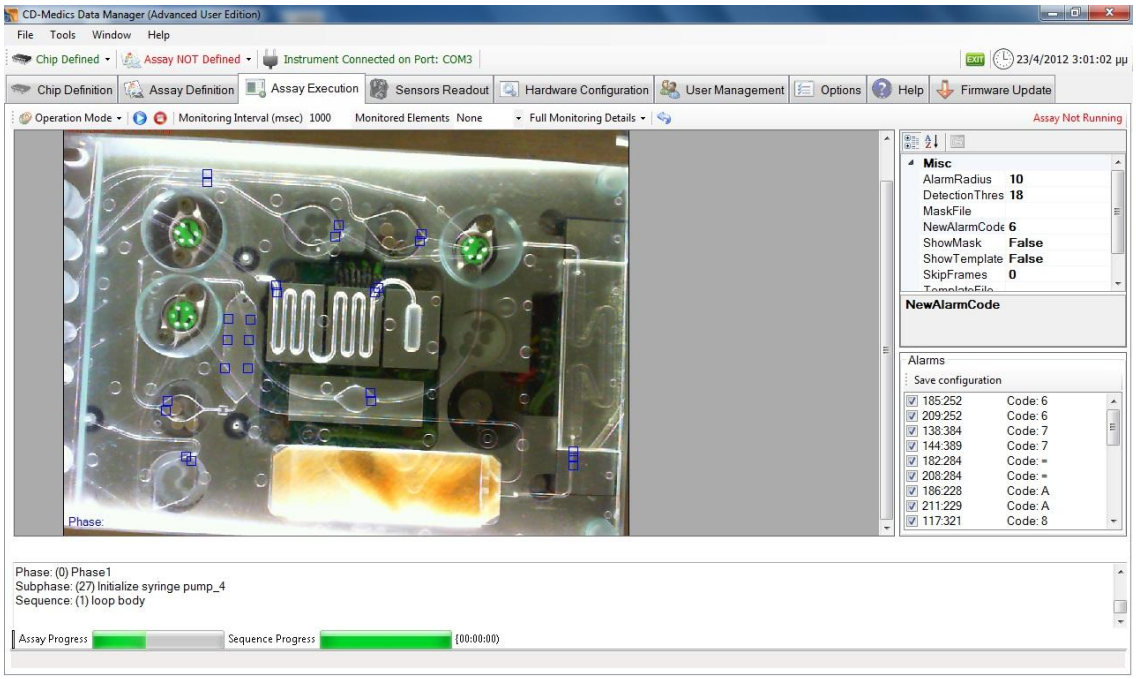

Figure 4 The machine vision fluidics control allows also for visual monitoring of the assay progress and insight into the operating instrument when in expert mode of the Data Manager Software

The integrated system of instrument, microsystems, control software and communications has been tested and validated

With respect to the communications aspects of the project additional security features have been incorporated in the software components. (e.g. device registration and authentication, message encryption, etc.). These have been integrated into the instruments Data Manager software and taking into account the network and security environment of the pilot test location. Testing of the entire communications system has been successfully achieved. Evaluation activities have also been undertaken including assessment of the user acceptance of the communication infrastructure, through human interaction and collection of associated feedback from IT personnel at the evaluation site. Within the same context the participants organized and supervised closed circuit testing of the results stream from the Data Manager of the instrument into a shadow Hospital Information System

An extensive validation of all assays, components, modules and integrated microsystems was carried out throughout the final period. In order to achieve objective validation, Partner FRCBS identified the Director of Quality Assurance, Marjatta Hirvonen, at the Finnish Red Blood Service as an independent reviewer of validation plans and results. A process for validation was agreed as outlined below.

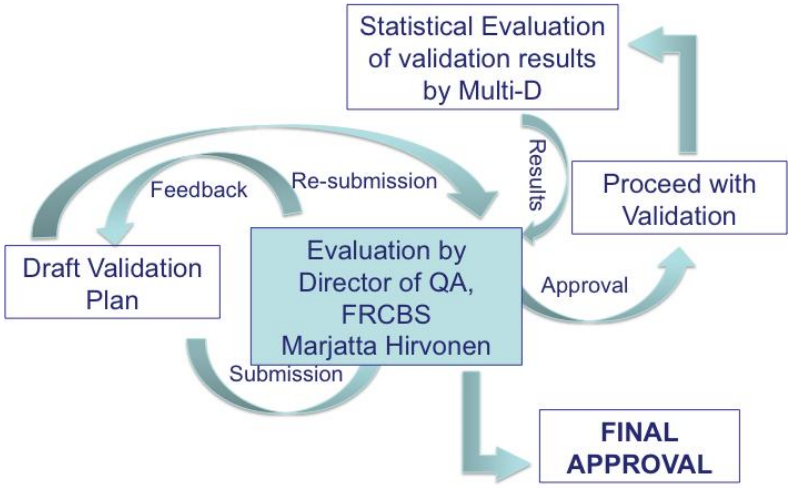

Figure 5 Outline of procedure for validation of all assays/components/modules/systems

Within the validation process assays (HLA and serology), microsystems and their sub-components as well as the integrated system were tested and validated using the agreed methodologies.

The e-learning tool for healthcare professionals has been further enhanced and updated and will be re-submitted for accreditation. The figure below shows the home page of the module.

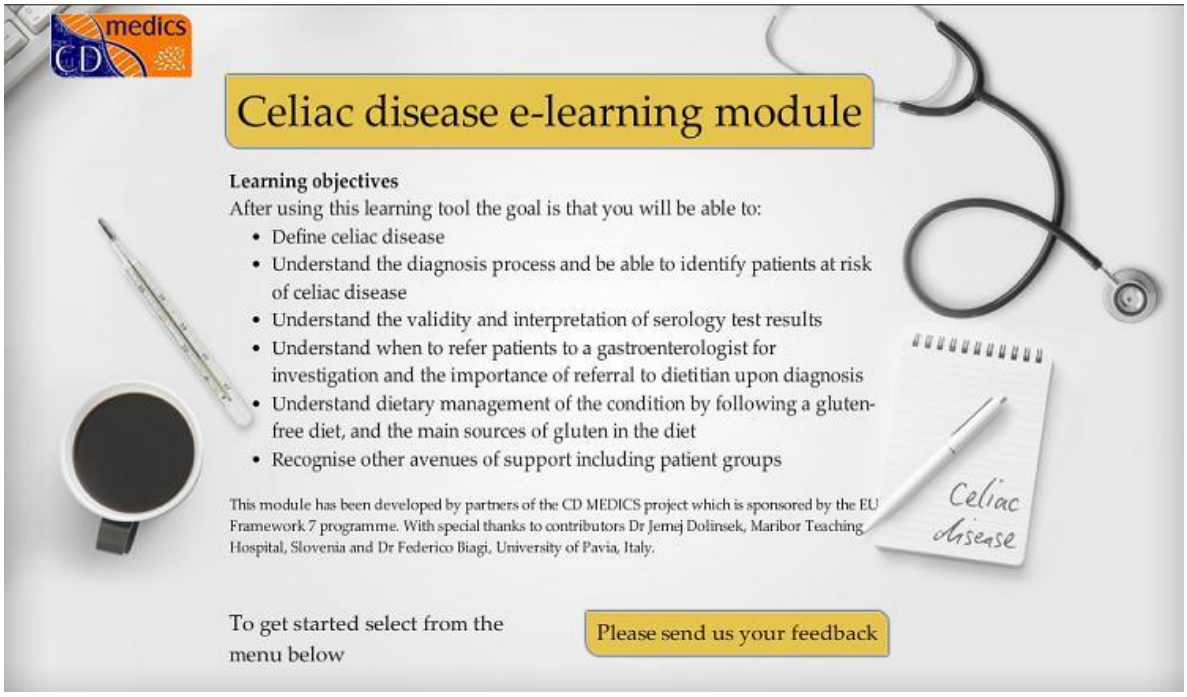

Figure 6 Online healthcare professional training tool

A number of training and roadshow events have been held (Malta, Riga and Kiev) which introduced the general public and healthcare professional to CD. The Malta meeting was a joint meeting with another FP7 project called MEDICEL.

Impact

The economic impacts of the results of CD-MEDICS are potentially very considerable with proposed technologies that will facilitate improved healthcare provision through fast and flexible point of care systems, to consumer devices. Expenditures for diagnosis represent generally less than 1% of the total health care expenditure so increased testing cannot significantly increase costs but it can significantly contribute to the quality of health care as it:

- Allows **earlier and more appropriate** and therefore less costly treatment
- Provides higher quality care **at the patient location**
- Helps to **rule out expensive treatments**
- **Reduces costs** of treatment of complications
- Potentially **shortens the length of hospital stay** by earlier identification of celiac disease

The project therefore aims to contribute to:

- Benefit the citizen by providing easier access to celiac disease healthcare
- Reduce the costs of healthcare provision by improved and timely diagnosis
- Creating market opportunities that would not otherwise exist, especially for SME's
- Economic benefits due to increased productivity, by reducing the impact of illness both on the individual and community.

- Greater academic knowledge in understanding disease pathogenesis, this will impact in the are of celiac disease as well as other autoimmune conditions

The project website can be found at www.cdmedics.eu and contains an overview of the project as well as all of the public dissemination materials.

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