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

MNBS 2012
The 6th Annual Concertation and Consultation Workshop of EC funded projects on Micro-Nano-Bio Systems

Landing in the real world: Creating a supply chain

National Centre of Scientific Research "Demokritos", Athens, Greece
3 - 4 May 2012

Ioanis Katakis,
Universitat Rovira I Virgili, Tarragona, Spain
• Contract No: 216031

• Instrument: Large Scale Integrating Collaborative Project

• Priority: FP7-2007-ICT-1

• Duration of 54-months, commenced 1\textsuperscript{st} February, 2008

• 20 partners from 10 Member states

• Total EU-financing: 9.5M€
Overview of CD-MEDICS

Healthcare problem: Coeliac disease is a genetically pre-disposed autoimmune disease provoked by ingestion of gluten and affects at least 1% of the European population and has serious consequences if un/mis-diagnosed.

- Approximately 85% of cases unrecognized
- Correct diagnosis can take up to 30 years
- Average Diagnosis Time is 11.7 years
- For every one CD sufferer diagnosed, there are 7 undiagnosed
Lack of diagnosis/misdiagnosis leads to:

• Unnecessary hospitalization and incorrect treatment with costly drugs
• Malabsorption and single or multiple nutrient deficiencies
• Growth retardation, delayed development, behavioural problems and poor educational achievement
• Anaemia, osteoporosis, fatigue, low fertility, or neurological defects
• A 10 fold increased risk of small intestinal lymphoma
• Standardised mortality rate of twice that of the general population, non-Hodgkin lymphoma being the main cause of death
• Reduction in quality of life with extensive negative economical consequences on a societal level

ALL OF THESE ARE REVERSIBLE/AVOIDABLE VIA DIAGNOSIS AND ADHERENCE TO A GLUTEN-FREE DIET (THE EARLIER THE BETTER!)
Mass screening is the only way to identify the majority of patients with coeliac disease.

• Serology testing alone is inadequate, as there are a number of false negatives, particularly with milder (Marsh I, II) enteropathy, as well as some false positives

• HLA-typing alone will also detect subjects who will not eventually develop coeliac disease.

• A combination of serology and HLA-typing is the **only** definitive way to screen for coeliac disease, with the combined detection allowing diagnosis of symptomatic, silent and latent coeliac disease patients, and the combination of these is expected to achieve a 100% specificity and sensitivity, with zero false-positives or false-negatives, as the results of one test support the other.
New guidelines recently published have changed the diagnostic criteria for diagnosis of coeliac disease from the previous golden standard of biopsy to a combination of HLA-typing, and detection of total IgA and IgA anti-tissue transglutaminase autoantibodies.

ESPGHAN guidelines for the diagnosis of coeliac disease in children and adolescents.

An evidence-based approach

System Specifications

CD-MEDICS Specification

- HLA Chip
- Sensors and Assay
- Serology Chip
- Patient
- Clinical
- Market
- Manufacturing
- Fluidics
- Interfaces
- Communications
- CD-MEDICS Instrument
## System Specifications

<table>
<thead>
<tr>
<th>End user requirements</th>
<th>Serology</th>
<th>HLA typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of microsystem test modules:</td>
<td>2 separate (1x HLA, 2x serology)</td>
<td></td>
</tr>
<tr>
<td>Chemical storage:</td>
<td>Wet chemical storage in external tank modules</td>
<td></td>
</tr>
<tr>
<td>Instrument weight:</td>
<td>2-5 kg</td>
<td></td>
</tr>
<tr>
<td>Test time (bleed to read):</td>
<td>&lt;= 15 min</td>
<td>&lt;= 25 min</td>
</tr>
<tr>
<td>Maximum disposable sales prices:</td>
<td>&lt;= 12 €</td>
<td>&lt;= 25 €</td>
</tr>
<tr>
<td>Maximum acceptable instrument sales price:</td>
<td>&lt;= 8000 € HLA+serology integrated</td>
<td></td>
</tr>
<tr>
<td>Number of instruments:</td>
<td>1 integrated</td>
<td></td>
</tr>
<tr>
<td>Sample:</td>
<td>Finger prick</td>
<td></td>
</tr>
<tr>
<td>Sample volume:</td>
<td>5-10 μl (plasma/serum)</td>
<td>15-25 μl (whole blood)</td>
</tr>
<tr>
<td>Sample introduction to the microsystem:</td>
<td>Luer system with closing cap</td>
<td></td>
</tr>
<tr>
<td>Application of sample:</td>
<td>Application to microsystem after placement within instrument</td>
<td></td>
</tr>
<tr>
<td>Sealing the blood sample prior to running an assay:</td>
<td>Yes, following biological safeness issues</td>
<td></td>
</tr>
<tr>
<td>Maximum CV:</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Probable users of the system:</td>
<td>General practitioner, nurse</td>
<td></td>
</tr>
<tr>
<td>Shelf life of the disposables needed:</td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td>Instrument Calibration:</td>
<td>Automated calibration</td>
<td></td>
</tr>
<tr>
<td>Processing of waste:</td>
<td>Stored on the microsystem</td>
<td></td>
</tr>
</tbody>
</table>
## System Specifications

<table>
<thead>
<tr>
<th></th>
<th>HLA Typing Chip</th>
<th>Serology Chip</th>
</tr>
</thead>
<tbody>
<tr>
<td>footprint</td>
<td>footprint microtiterplate (127.8 x 85.5 mm)</td>
<td></td>
</tr>
<tr>
<td>functional modules</td>
<td>DNA isolation</td>
<td>sample injection</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>sample preparation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plasma separation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sample purification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>target detection</td>
</tr>
<tr>
<td>fluidic functions</td>
<td></td>
<td>fluid storage; fluid driving &amp; venting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>metering &amp; flow sensing; mixing &amp; dissolving</td>
</tr>
<tr>
<td>location of functional elements</td>
<td>fixed location for all functional elements on both chips</td>
<td></td>
</tr>
<tr>
<td>fluid storage requirements</td>
<td>separate storage of dried reagents on-chip and liquid stored reagents off-chip</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Storage shelf life of 12 months</td>
</tr>
<tr>
<td>sample injection</td>
<td></td>
<td>via blood collecting capillary</td>
</tr>
</tbody>
</table>
Microsystem concept with standardized landscape

HLA Chip

Serology Chip
Serology microsystem

- Waste chamber
- Serum generation
- Sample inlet
- Metering chamber
- Storage tanks
- Electrode array
- Dilution chamber
- Metering channel
- Storage dry reagent
What do we need to detect?

**HLA TYPING**

- **Motivations:** ca. 100% of Coeliac disease affected patients carry DQ2 (ca. 95%) or DQ8 (ca. 3%).
- **Targets:**

```
<table>
<thead>
<tr>
<th>DQ2 cis</th>
<th>DQB1</th>
<th>DQA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201</td>
<td>0201</td>
<td></td>
</tr>
<tr>
<td>0301</td>
<td>0505</td>
<td></td>
</tr>
<tr>
<td>0202</td>
<td>0202</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DQ2 trans</th>
<th>DQB1</th>
<th>DQA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0302</td>
<td>0302</td>
<td></td>
</tr>
</tbody>
</table>
```

**Targeted Alleles:** Red & Blue associated alleles; Orange suspected associated alleles

**SEROLOGY**

- **Motivations:** Large number of patient samples tested for various autoantibodies and IgA deficiency
- **Targets:**
  - Total IgA antibodies (screening for IgA deficiency)
  - IgA anti-tissue transglutaminase antibodies
  - IgG anti-gliadin / deamidated gliadin antibodies

**ISOLATION OF ANTI-HUMAN ANTIBODIES & QUANTITATIVE DETECTION**
HLA Typing: Direct detection of PCR product

**AMPLIFICATION:**

A) Forward primer
B) Reverse primer
C) Fishing sequence
D) PCR stopper
E) Tail 1
F) Tail 2

**DETECTION:**

P201230120, “Oficina Española de Patentes y Marcas” entitled “Cebadores especiales para la reacción en cadena de la polimerasa”

Filed 30th January 2012
HLA typing of real samples (multiplexed PCR products).

Hybridisation time (2 minutes), reporting label capturing time (2 minutes), assay temperature 37 ºC.
## HLA Typing: Real sample analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>ELECTROCHEMICAL TYPING</th>
<th>REFERENCE TYPING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3b</td>
<td>4y</td>
</tr>
<tr>
<td>8087</td>
<td>+/-</td>
<td>(0.93)</td>
</tr>
<tr>
<td>8092</td>
<td>-</td>
<td>(0.1)</td>
</tr>
<tr>
<td>FRCBS 25</td>
<td>-</td>
<td>(0.56)</td>
</tr>
<tr>
<td>FRCBS 25 (bis)</td>
<td>-</td>
<td>(0.36)</td>
</tr>
<tr>
<td>FRCBS 12</td>
<td>+</td>
<td>(3.31)</td>
</tr>
<tr>
<td>FRCBS 12 (bis)</td>
<td>+</td>
<td>(1.93)</td>
</tr>
<tr>
<td>FRCBS 20</td>
<td>+</td>
<td>(1.47)</td>
</tr>
<tr>
<td>FRCBS 20 (bis)</td>
<td>+</td>
<td>(5.26)</td>
</tr>
<tr>
<td>FRCBS 31</td>
<td>-</td>
<td>(0.59)</td>
</tr>
<tr>
<td>FRCBS 31 (bis)</td>
<td>-</td>
<td>(0.28)</td>
</tr>
</tbody>
</table>

Summary of the HLA typing of real samples (multiplexed PCR products).

Assay complete in < 5 minutes!
DNA sensors completely stable >2 years @ 4°C
Antibody detection

- Electrochemical immunosensor for detection of autoantibodies. 5 minute sample incubation, followed by 5 minute reporter antibody incubation and 1 minute product generation

- Detection of total IgA, anti-tTG and anti-gliadin
Serology: Real patient samples

Anti-tTG

Anti-gliadin

Total IgA

Non-deficient

IgA Deficient
Accelerated Thermal Arrhenius stability studies show that antigen coated electrodes stable > 2 years at 4°C
Stability of lyophilised mediator-substrate

Stable > 2 years at 25°C in powder/liquid form
Microelectrode arrays

- Photolithography: 52€/array
- Screen-printing: 1.2€/array
- Electrolytic Au on PCB: 0.06€/array
Lifecycle of microsystem prototype

- High complex concepts
- Microsystem designs
- Prototype fabrication
- Mass fabrication
On-chip, 2-T PCR

On-chip PCR, 35 cycles in 5 minutes

-Trials with DBQ-Primers and SSP-Primers
-Amplification successful in thermocycler, and on-chip
• Liquid reagents stored in tanks:
  • Long term stability has been demonstrated of reporter antibodies/DNA molecules
  • No water loss observed with sealed tanks stored at 4°C and 22°C

• Lyophilised/dried reagents stored on chip:
  • Long term stability has been demonstrated of lyophilised PCR mastermix stored at 4°C and 22°C
  • Long term stability has been demonstrated of dried lysis reagents and beads for DNA isolation stored at 4°C and 22°C
  • Long term stability has been demonstrated of mediator/substrate for HRP enzyme reporter at 4°C and 22°C dried on chip and at 4°C stored in black tank in liquid format
Injection molded microsystems

HLA Typing

Serology
Lifecycle of the instrument prototype

1st version of the slot
Electronics
D.M. SW 1 user group

Y1
Concept
CAD specs
Block Diagrams
Mockup Screens

Y2
1st instrument prototype
3 lightweight versions
Instrument Companion SW

Y3
Complete instrument
For Validation

Y4
1st instrument Prototype integrated, ...
Integration meeting, Athens, June 2010
Simplified processing

Annotation of the microsystem

Control using a camera
Microsystems within instrument
Camera control of fluidics

20110100390 "Integrated System for the Visual Control, Quantitative and Qualitative Flow Measurement in Microfluidics". 2011 – Greek patent application – European to be filed July 2012
Flexible fluidics
Flexible Instrument

Mounting Block €69.47

Motor & Mixer Mounting Plate €35.-

+ 1 Cable + a few mounting screws...

Hardware Configuration Editor

Protocol Editor
2nd Generation Instrument
Communication with HIS: HL7

Hospital Information System / Patient Administration System

Laboratory Orders / Observations

Laboratory Orders / Observations

Laboratory Workstation / Orders Management System

CD MEDICS Instrument

Demographics Requests/ Replies
• Assumption:
  – 1,000 pieces in a period of three years
  – Target cost for distributors:
    • €4,500 (Instrument),
    • €5 (Injection molded microsystem with reagents & electrodes – serology)
    • €8 ((Injection molded microsystem with reagents & electrodes – HLA typing)

• Initial investment necessary (molds etc.)

• Estimated time to market: 2 years
  – only CE certified (not complete IVD certification; clinical trials excluded)
Further diseases

- HASHIMOTO’S THYROIDITIS
- RHEUMATOID ARTHRITIS
  - 28 real serum samples from RA patients
  - 28 DRB1 Type 11 DNA samples – HLA typed
  - Rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) purchased for detection of autoantibody
  - DNA probes will be ordered
- ANKYLOSING SPONDYLITIS
  - > 20 HLA Typed DNA samples
Health care professional interactive training tool – on the way to accreditation by European Accreditation Council for Continuing Medical Education

- Multiple roadshows
- Trade fairs (Compamed, Analytica, Biotechnica)
- Pamphlets for doctors (18 languages to date – plan to increase)
- External and internal newsletters
- Medical exchange visits
- Exchange visit between partners
**Celiac disease e-learning module**

**Learning objectives**
After using this learning tool the goal is that you will be able to:

- Define celiac disease
- Understand the diagnosis process and be able to identify patients at risk of celiac disease
- Understand the validity and interpretation of serology test results
- Understand when to refer patients to a gastroenterologist for investigation and the importance of referral to dietitian upon diagnosis
- Understand dietary management of the condition by following a gluten-free diet, and the main sources of gluten in the diet
- Recognise other avenues of support including patient groups

This module has been developed by partners of the CD MEDICS project which is sponsored by the EU Framework 7 programme. With special thanks to contributors Dr Jernej Dolinsek, Maribor Teaching Hospital, Slovenia and Dr Federico Biagi, University of Pavia, Italy.

To get started select from the menu below

Please send us your feedback
Application submitted to the European Accreditation Council for Continuing Medical Education (EACCME)

Ensure access to quality CME activities and securing European exchange of CME credits for the medical specialists in Europe

- Expected educational outcomes
- Level of evidence
- No conflict of interest or bias
- Active method of learning
- Self-assessment
A compact multichannel potentiostat for real-time electrochemical biosensing applications, I. Ramfos, N. Vassiliadis, K. Efstathiou, A. Fragoso and C. K. O’Sullivan, Submitted to IEEE

Antibodies to wheat high molecular weight glutenin sub-units in 1 patients with coeliac disease, Julia Ellis, Pablo Lozano-Sanchez, Carmen Bermudo, Tanja Šuligoj, Federico Biagi, Paola I Bianchi, Gino R Corazza, Annalisa De Silvestri, Enzo Bravi, Ioannis Katakis, Ciara K O’Sullivan and Paul J Ciclitira, International Archives of Allergy and Immunology, Accepted March 2012


Other dissemination activities:

> 50 workshops and training activities

> 250 articles in newspapers, webpages, magazines…..

Videos related to CD-MEDICS uploaded to YouTube (http://www.youtube.com/user/CDMEDICS), >8500 views!
Validation

- Validation of all discrete microsystem modules (e.g. serum dilution, cell lysis, DNA isolation, PCR, sensor detection) has been/is in final stages of completion completed using real clinical patient samples

- Communication of assay results to electronic health record has been demonstrated in house at UMC Maribor

- Overall system ready to be implemented at UMC Maribor for validation using as many real patients as possible (30 – 100) in real clinical setting in period June-September 2012
Project products for commercialisation

- Commercialisation agreement has been reached by Eurospittal-MFCS-Micro2Gen-URV-Innotrain for post-project commercialisation of overall system by Eurospittal
- Innotrain-URV – double stranded double tailed PCR product – application to pharmacogenomics, microarrays
- Micro2gen-URV-MFCS – research tool-box of electrode arrays – multichannel potentiostat – microfluidics
- TATAA – multiplex real time PCR for SSO HLA typing
- Multi-D – e-learning module
The CD-MEDICS Consortium
The CD-MEDICS integrated project is partly funded by the European Commission (IST-2007-1-216031).

www.cdmedics.eu