EMAIL SUBJECT:
INTOPSENS NEWSLETTER 3 - **Recent progress in SEPSIS diagnostic test development.**

Introduction:
This newsletter overviews the recent results in the European INTOPSENS Project.
Intopsens develops nanophotonic biosensors for the fast identification of septic bacteria strains and their antibiotic resistance from whole blood.

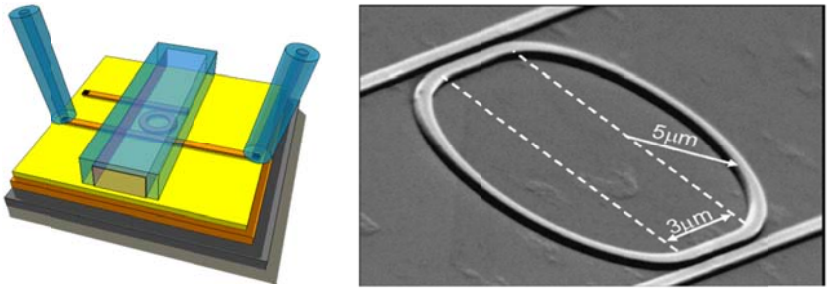
Highlights of technology developed in the last 12 months:

Year two of the project has been very successful, having achieved most of its objectives and technical goals, as well as producing 12 peer review articles and 4 patent applications!

- **In cartridge and materials aspects** a new, generic, thiol-ene based polymer system has been developed, specifically for lab-on-a-chip systems. The new polymer systems combines all benefits of PDMS soft polymer replication (ease of handling, fast prototyping) but circumvents its drawbacks, allowing for controllable surface chemistry properties, e.g. photopatternable grafting on the polymer surface has been demonstrated, and controllable bulk material behavior, e.g. both stiff materials, but also elastomeric variants have been demonstrated. These results were for the first time presented as an oral presentation at microTAS 2010 conference in Groningen, the Netherlands.
- **Bioassay:** the development and first-round validation of hybridization probes on the Prove-it™ TubeArray system has taken place for both UA and MD assay. We have also performed the first successful on-chip PCR, demonstrating the amplification of 300bps e-coli genomic material.
- **In photonics** a biosensor consisting of two cascaded ring resonators based on the Vernier-effect was implemented in silicon-on-insulator and its sensitivity determined to be as high as 2169nm/RIU in aqueous environment. Photonic crystals employing the system BSA/antiBSA demonstrate a surface mass detection limit below 2.1 pg/mm^2 and a total mass detection limit below 0.2 fg
- **In DNA biosensing** single-stranded DNA has been recognised with ring resonator sensors (APTES-chemistry) and photonic crystal sensors (ICPTS-chemistry). Photonic crystal sensor limit of detections for double-strand DNA detection and DNA hybridisation are 22.2 and 19.8nM respectively.

Overview of Project:

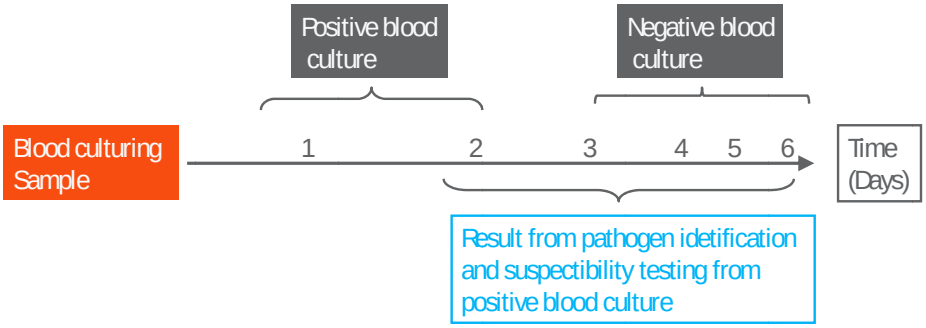
Intopsens (project number 223932) is an EC 2.6M€ funded FP7 ICT call 2 multidisciplinary project (1/9/8 - 31/8/11), involving the emerging fields of photonics structures, electronics, fluidics and bio-chemistry, to contribute to the development of high value sensor technology. It integrates two label-free biomolecular recognition photonic sensor technologies with sensitivities below 1ng per ml, near to or beyond state-of-the-art in label-free integrated optical biosensors, with novel coupling technology that will permit very high integration of hundreds of sensing areas on a 1mm^2 photonics chip.



Schematic of biosensor based on ring resonators in SOI

The novel diagnostic technology of the InTopSens device has the potential to be fast and easy to use, making routine screening or monitoring of bacteria more cost-effective and will be demonstrated as a rapid diagnostic test for sepsis at 'point of care'.

Sepsis is a life threatening medical condition that claims the lives of ~146000 people per year in the EU and whilst it deteriorates hour by hour current diagnostics are too slow to make an impact on the all-important first hours of patient treatment. The InTopSens device will detect the bacteria and determine its antibiotic resistance profile with an hour enabling targeted antibiotics treatment to both benefit patient recovery rates and prevent an increase in bacterial antibody resistance.

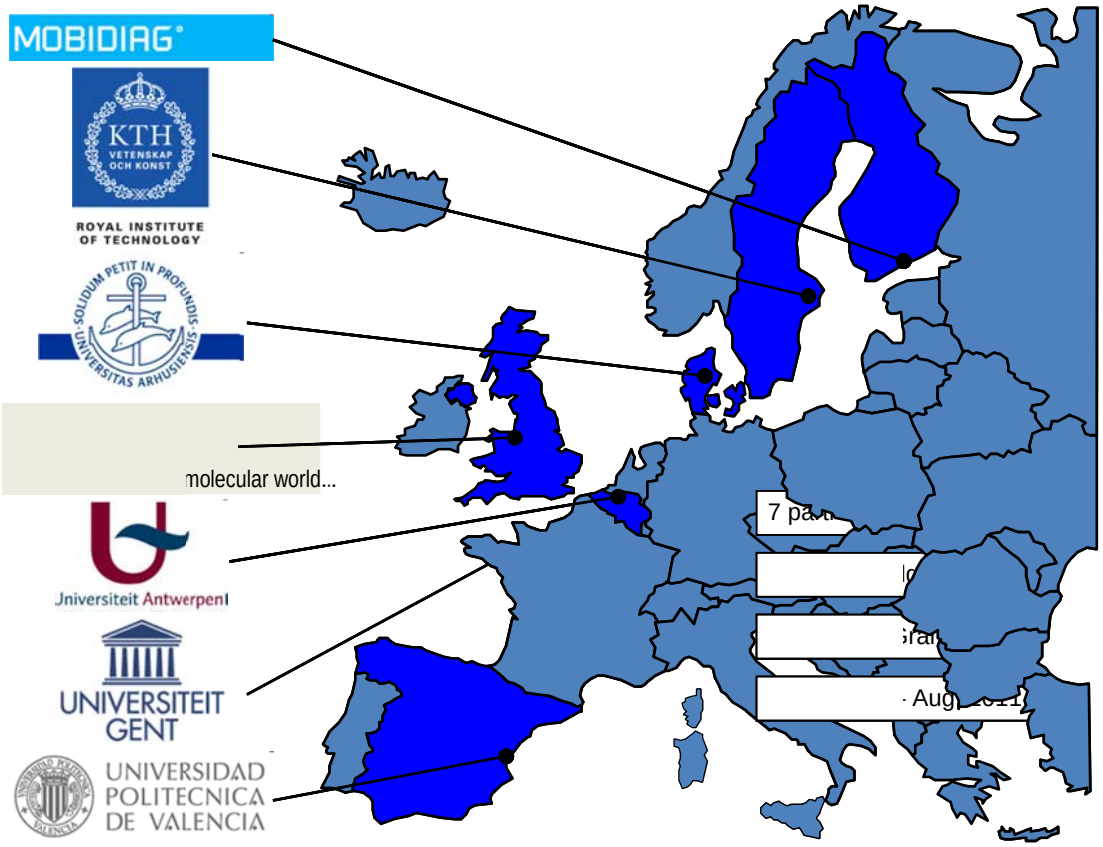


Typical time frame of the sepsis diagnostics Gold Standard that is based on microbiological methods.

Some 120 sensing areas/datapoints are needed to identify this profile and the very high integration of up to 250 assays on a 1mm^2 chip will permit higher sensitivity/selectivity for the same bacteria for or simultaneous identification of other bacteria. A final prototype consisting

of a packaged biochip will be used on clinical samples in order to detect the sepsis bacteria and determine their resistance to antibiotics.

The project partners, consisting of both SME and academic institutes, are distributed amongst 6 European countries and are fully complementary for such a project. The project's setup and partners' background ranges from physics through to microfluidics and to medical science. This multidisciplinary mixture has the potential to work as a key accelerator for novel, ground-breaking scientific development. The management structure of the consortium is designed to ensure the maximum participation between partners from the disparate backgrounds without the need for each partner to become experts in each other's fields.



Partner feature:

Mobidiag Ltd

www.mobidiag.com

Mobidiag Ltd is a Finnish biotech company located in the middle of the interactive Meilahti medicine and health care campus in Helsinki. The company is founded in 2001 and it is specializing in rapid diagnostics of infectious diseases. The core competency is the development of reliable diagnostic assays by combining the know-how of clinical questioning and assay content design with world leading platform and software technologies. This unique approach results in improved diagnostic processes and increased customer value.

Mobidiag has developed and provided unique and innovative microarray-based Prove-it™ technology for early and accurate diagnosis of severe infections caused by life-threatening pathogenic microbes. Currently two microarray platforms are available; Prove-it™ TubeArray is a compact single array while Prove-it™ StripArray is a high throughput microarray system, capable of analyzing 96 patient samples in one run. Pathogen identifications are objectively and automatically reported by the proprietary bioinformatics software Prove-it™ Advisor.

Currently, Mobidiag has two CE-marked products for *in vitro*-diagnostics; Prove-it™ Sepsis and Prove-it™ Herpes. Prove-it™ Sepsis test identifies over 64 sepsis-causing bacteria from positive blood culture in a single assay. The evaluation study against the conventional culturing method, conducted in two major European hospitals, shows astonishing 95 % clinical sensitivity and 99 % specificity for the assay. Prove-it™ Herpes assay is for clinical diagnostics of human herpesviruses. The assay enables simultaneous identification of seven human herpesviruses from cerebrospinal fluid samples. The evaluation study, conducted against PCR-based reference methods, shows respectable 98 % clinical sensitivity and 90 % specificity for the assay. Mobidiag has also launched two research-use-only products: Prove-it™ Sepsis application for Bone and Joint infections and Prove-it™ Sepsis application for Fungi. Prove-it™ tests contribute to faster and more evidence-based patient management and thus, positive outcomes for seriously ill patients.

Mobidiag continues to develop and provide rapid and accurate tests that reliably detect pathogenic microbes and the current Prove-it™ tests are important milestones on that road.

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Bioassay development feature:

In the final InTopSens device the rapid and sensitive microbial identification together with antibiotic resistance detection plays a major role. Mobidiag has been actively taking part of the development of the bioassay together with University of Antwerp. The pathogen panel of the bioassay includes the most common sepsis-causing bacteria, *i.e.*, *Escherichia coli, Staphylococcus aureus* and *Staphyloccocus* sp. In addition to the identification of bacterial species, the detection of methicillin and fluoroquinolone resistance markers will be included in the assay. Detecting bacteremia would require development of highly sensitive and robust assay, the number of detectable bacterial cells can vary from ten to millions in millilitre of blood, which challenges the development work.

In the IntopSens project, we have standardized the developmental settings using the similar amplification and detection methods and the same well-characterized sample material. The University of Antwerp has been characterizing and providing bacterial species and DNA samples for the wet-lab experiments and Mobidiag has supervised and provided the use of Prove-it™ microarray platform for the development of microarray content and result reporting step. This approach has allowed us to share the work load and compare the results reliably. We have shown the proof-of-concept for the sensitive bacterial identification. Now we continue towards adding the resistance markers and selecting the high quality reagents for the final bioassay. This is a good example of the collaboration which has been remarkable between the InTopSens partners. Effective collaboration between companies and academic groups not only gives great opportunities for learning from each other, but also ensures that the final outcome of the project is able to fulfill the need of the market. For more details about the project please visit the website www.intopsens.eu where both the bioassay and all the other aspects of the project are described.