The Intopsens consortium...

- will develop a label-free biosensor for the point of care identification of sepsis bacteria strains and their antibiotic susceptibility from whole blood
- will create disposable lab-on-chip cartridges with integrated microfluidic sample preparation and ultrasensitive photonic transducers, as well as a bench-top readout system
- focuses on a rapid solution (<1h) with little hands-on time, so as to be used at point of care (PoC) in an intensive care unit by paramedics and General Practitioners
- consists of five research centres and two industry partners from six European countries
- is supported during 2008-2011 with 2.6 Million Euro by the European Union through its Seventh Framework Programme.
Sepsis as a condition and current diagnostic methods

Sepsis is a serious medical condition and claims ~146,000 lives/year in the EU and an associated EUR 7.6 billion/year health care costs. Bacteremic patients with delayed or ineffective antibiotic treatment in the first 72 h experience a significant, approximately twofold increase risk of fatal outcome. This untoward situation is not uncommon and may affect 15% to 40% of bloodstream infections (BSI) cases depending on the setting. On the other hand, the fear not to provide effective empirical therapy often leads to “over-treatment” with multiple broad-spectrum drugs, which in turn contribute to selecting for more resistant strains in that population. Microbiological tests currently available for the diagnosis of bloodstream infection rely on automated blood cultures systems, which typically detect bacteraemia within 24 to 48 h, followed by bacterial identification and antimicrobial susceptibility testing which may take another 12 h to 36 h to be completed. Fast (2-6 h) bacterial identification methods are available on automated biochemical test platforms (such as Vitek 2 and Phoenix systems) but require a preliminary 18 h subculture on solid media from positive blood culture for reliable results.

Other technologies like multiplex real-time PCR and DNA microarray are still under development. Among these techniques, Roche has developed PCR techniques applicable on blood samples without culture. The difficulty, here is that nucleic acid amplification of a non-cultured sample tends to provide too many false positive results and fails also to distinguish clearly between infection-causative and colonizing or contaminating organisms.

Thus, faster, broad range bacterial identification and resistance detection systems for positive blood cultures, or samples subjected to limited culture, are needed to improve the appropriateness of early antimicrobial therapy of bloodstream infection.

Lab-on-chip technology for bacterial isolation

Intopsens is developing a state-of-the-art diagnostics platform via an integrated microfluidic sample preparation technique capable of rapid bacteria isolation from whole blood. This integrated Lab-on-a-Chip platform involves capturing of intact bacteria from relatively large blood volumes ~5 mL, pre-concentration, purification and cell lysis to extract the genomic material for on-chip PCR amplification and sensing.
Cell lysis releases the bacterial genomic material, DNA, available for the sub-sequential on-chip PCR amplification. During the PCR, selected genomic targets representing characteristic DNA sequences for bacterial species identification and resistance profiling are exponentially copied. The genomic targets are further identified on the photonic sensing array. The array contains tens of sensing sites, each specifically functionalized to identify a certain genomic target. This hybridization technique allows the discrimination of very small variations in the genome. Thus, in addition to the bacterial species identification, it is also possible to detect genes that are responsible for antimicrobial drug resistance. For example, the bacterium *Staphylococcus aureus* harboring the methicillin resistance gene is the superbug MRSA.

**Photonic transducers provide optical readout**

Two types of photonic transducer arrays are being developed: photonic wire based sensors and photonic crystal based sensors. Both are fabricated using the same deep-UV technology that is used for electronic chip fabrication, which opens the route towards cheap, disposable devices. The photonic wire sensors are based on ring resonators, typically 10 micrometer across. The photonic crystal sensors are even smaller, but as sensitive, and are based on single-mode defects waveguides consisting of one missing row of holes in a planar photonic bandgap. When light is coupled into the sensors, they resonate at a certain wavelengths. Each sensing site has a specific capture zone where the PCR product hybridises with attached genetic probes. The resonance frequency changes as biomaterial attaches to the surface, thus providing the identification of the DNA sequences. Due to the small size, many sensors can be put next to each other on a single photonic chip. The sensitivity of the sensors, especially of the photonic crystals, may come close to the single-molecule limit. Technology is also being developed to couple in and read out light from the entire biosensor array at once.
The Intopsens Consortium

KTH – the Royal Institute of Technology The Microsystem Technology Lab is a leading MEMS and microfluidics group. The Cell Physics Lab researches the interface between cell biology and physics, including cell-on-chip systems. Contact: Prof. Wouter van der Wijngaart, wouter@ee.kth.se, www.ee.kth.se/mst

University of Aarhus The iNANO centre and AGSE are involved through the nanophotonics group, which is performing development of silicon photonic crystal waveguides for bio-sensing. Contact: Prof. Martin Kristensen, mk@phys.au.dk, URL: www.inano.dk

Farfield Group Ltd is a UK instrumentation company specializing in measurement of conformational changes in proteins for bioanalytical purposes. Farfield’s technology measures sub atomic dimensional changes in proteins implicated in a host of disease processes and is used to study the disease mechanism and drug candidates to inhibit it. Contact: Dr. Gerry Ronan, gronan@farfield-group.com, www.farfield-group.com

University of Antwerp is involved through its Department of Microbiology, and through the Laboratory of Clinical Pathology at the University Hospital. Contact: Prof. Dr. Herman Goossens, herman.goossens@uza.be, www.ua.be

Mobidiag Ltd. is a Finnish biotech company specializing in rapid diagnostics of infectious diseases. Mobidiag develops and provides unique and innovative microarray-based Prove-it™ technology for early and accurate diagnosis of life-threatening pathogenic microbes causing severe infections. Contact: Dr. Antti Vuolanto, antti.vuolanto@mobidiag.com, www.mobidiag.com

Gent University - the photonics group at Ghent University is one of the main players in the field of silicon nanophotonics Contact: Prof. Peter Bienstman, Peter.Bienstman@UGent.be, photonics.intec.ugent.be

Universidad Politécnica de Valencia The Nanophotonics Technology Centre performs R&D on photonic technologies and systems for telecom and signal processing. The Signal and Measurement in Chemistry group researches biosensing in life sciences. Contact: Assoc. Prof. Jaime Garcia, jaigarru@upvnet.upv.es; www.ntc.upv.es

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