Rapid Aptamer based diagnostics
for bacterial meningitis

RAPTADIAG
Rapid Aptamer Based Diagnostics for Bacterial Meningitis
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Research for the benefit of SMEs

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Contents

4.1a. Executive Summary

The RaptaDiag project was centred on the development of a fast, easy-to-use, inexpensive diagnostic test for *Neisseria meningitidis* (*aka meningococcus*) and *Streptococcus pneumoniae* responsible for bacterial meningitis (BM) which is on the WHO Top-10 list of lethal infectious diseases.

The objectives were to develop a sensor with in vitro synthesised DNA based receptor molecules, *Aptamers*, with a high specificity towards these organisms, to be used in three different sensors. One was an evanescent field biosensor (EVA) already in pre-production, while the other two based on micro-resonators and liquid crystals respectively were more experimental to be developed fully within the context of the project.

The Eva-biosensor was to be adapted to use aptamer sensors and to prove applicable to microbial detection. The novel micro-resonator sensors (MRS) based on aluminium nitride piezo electrics and the liquid crystal sensors (LCS) had to prove their potential in microbial detection, eventually detecting the target organisms using aptamers. The three sensors are mutually complementary:

- The EVA-Biosensor has become a fully commercially available multiplex sensor technology and proven for rapid (10min) and quantitative immunoassay tests. It has proven applicable to all tested of antigens (proteins, microbes, cells etc.), in any kind of sample (serum, water etc), with any kind of bioreceptor.
- The MRS have pushed detection levels to an unprecedented level of 4pg in liquids.
- LC sensors have proven their potential as extremely inexpensive and simple detectors of microbes

The consortium managed successfully to develop aptamers against of the fibronectin-binding protein (PavA) and QuinolinateSynthetase (NadA) specific to *S. pneumoniae* and *N. meningitidis*.

During the project the EVA-Biosensors have become a fully commercial product providing equivalent results to ELISA testing, but in a fraction of time and using a fraction of sample volume. The proven extreme sensitivity of the MRS have the potential to detect trace amounts of proteins with a very high specificity in all matrices, while the LCS have shown their potential for a rapid portable detector for microbes in aqueous solutions or bodily fluids.

All partners are collaborating, and open to new collaborations, in extending the application of the developed sensors to new PoC, environmental and public health contexts.

4.1b. Context and Objectives

The original objective of this project was to develop a fast, easy-to-use, inexpensive diagnostic test for *Neisseria meningitides* (*aka meningococcus*) and *Streptococcus pneumonia* responsible for bacterial meningitis (BM) which is on the WHO Top-10 list of lethal infectious diseases.

The new diagnostic tests were intended to be faster (minutes rather than hours or days) and cheaper (euros rather than several 10s of euros) than the currently available technologies. The test will address the clinical need for a diagnosis of these diseases with a high degree of morbidity, reducing the possibility of misdiagnosis, and abuse of antibiotics.

Microorganism recognition was to be achieved by the use of novel ***aptamer*** receptors rather than conventional antibodies. These aptamer receptors served as basis for all proposed diagnostic tests. Aptamers are short single stranded DNA/RNA molecules, which by intra-strand pairing of the nucleic bases undertake a 3-dimensional structure which is then selected based on its high affinity and specificity towards the desired antigen or target.

Aptamers show a number of advantages over antibodies: lower development and production costs and especially higher stability in non-ideal storage conditions. Furthermore, the low unit price of aptamers allows for a high number of receptors to be deposited on the active area of the sensor – thus increasing the sensitivity without compromising the total sensor cost. Two new highly specific aptamers targeting *N. meningitides* and *S. pneumonia* were to be developed during the project.

The consortium employs three different sensor technologies (Figure 1) towards the diagnostic tests. All these use aptamers as common receptor. The first technology was the commercial evanescent biosensor (EVA-technology -core sensor technology of partner three, the SME Davos Diagnostics) chosen for delivering rapid quantitative results. Two more experimental technologies were included for developing a rapid test at significantly lower cost, *i.e.* a microacoustic-resonating sensor (MRS) and liquid crystal based sensor (LCS).

Each of the three sensors approaches has their own advantages:

- The evanescent biosensor technology "*EVA-Biosensor*" represents a forefront, yet commercially available multiplex sensor technology and proven for rapid (10min) and quantitative immunoassay tests. The Eva-Biosensor provided a guarantee that at least one commercial product would benefit from this project.
- The microacoustic-resonating biosensor (MRS) technologies carry a high density of receptors, having the potential to detect the binding of one microorganism alone, which is the ultimate detection limit.

- The liquid crystal sensor (LCS) open the way for an exceedingly simple and inexpensive detection method, with either visual (without the need for *any* instrumentation!) or simple optoelectronic inspection with miniature readers or even mobile phone cameras

Both MRS and LCS employ direct, label-free, detection, which allows for the use of cheaper receptors for the specific detection. Low cost detection technology will be the target for both technologies.

Like the EVA-Biosensor, the experimental low cost technologies will be developed with a single use disposable diagnostic chip and a portable detection unit. For LCS qualitative diagnostic results will be possible with simple visual inspection - without the need of any instrumentation, leading to very simple and very low-cost diagnostics.

The principal **scientific-technological objectives** can be summarised as follows:

- development of new aptamers for the detection of *Neisseria meningitides* and *Streptococcus pneumonia* (Months 18 and 30)
- adaptation of aptamers to the EVA-Biosensor technology (Month 12)
- development of electronic detection based on resonant piezoelectric mass sensors with aptamers (Month 12 and 30)
- development of liquid crystal based sensor with aptamers (Months 18 and 30)
- adaptation of the new sensors for industrial production (Month 36)

It has to be emphasised that all these objectives have actually been met, with the exception of the last one, although the aptamer objectives experienced some delay. This last objective was possibly too ambitious at the onset of the project, given that the two new technologies have been developed from scratch in the responsible institution. Thus the technology readiness levels of the two new technologies must be today considered to be between 4 and 5.

Aptamers have been previously tailored for a number of bacteriological pathogens and the original partner Bioapter S.L. was one of the main established providers of commercial microorganism specific aptamers (*Legionella pneumophila* and *Salmonella spp*.). Available *Salmonella spp.* and *L. pneumophila* aptamers have been used in the project as test receptors for the development of the aptamer based EVA-Biosensor device at DDX as well as the UPM devices. Both whole cell and recombinant proteins from the pathogen surface were targeted for the aptamer development.

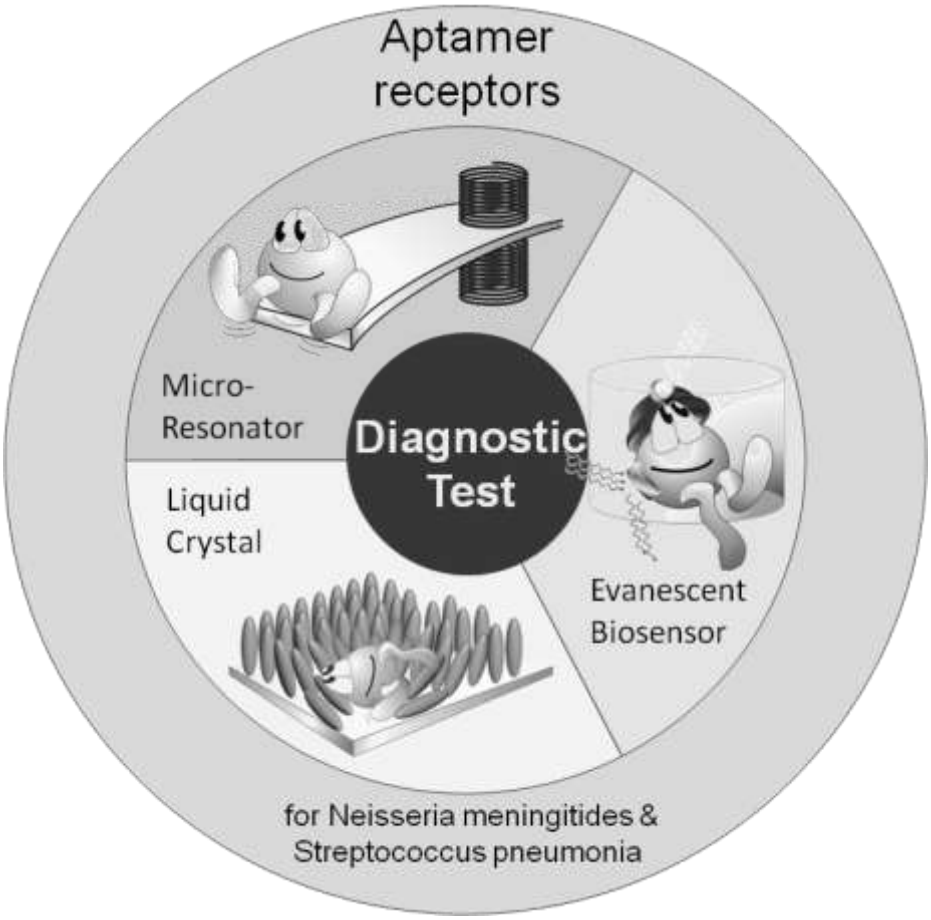


Figure 1: Sketches of the three proposed diagnostic test technologies using the novel specific aptamers to bind bacteria: In microresonators the bacteria causes a change in oscillation frequency. In liquid crystal sensors (LCS), the bacteria cause disruption in the alignment layers. In evanescent excitation (EVA), a secondary reporter receptor labelled with a fluorophore reports the binding of the target organism

DDX has commercially available sensor chips, "EVA-Chips", and readers, "EVA-Readers", employing conventional antibodies, along with its expertise in surface coating and buffer composition. Like conventional ELISA technology, the EVA-biosensor requires a secondary, potentially less specific, reporter receptor labelled with a fluorophore to bind the target. For the readout, the EVA-Chips are illuminated with a laser using evanescent excitation. *Unlike* ELISA only the reporter molecules bound to targets at the bottom of the well will get excited by the evanescent field from the interrogating light source, which reduces the false positives (originating from the bulk solution), eliminates the washing steps and reduces the signal background. The important tasks are the adaptation of the EVA-Biosensor technology to use aptamers, the determination of the binding protocol, and evaluating the potential use of aptamers as reporter molecules.

The adaptation of EVA-Biosensor technology to use aptamers and to target pathogens, present a direct commercial value for this SME.

Piezoelectric and liquid crystal devices and their manufacturing have been the core activities of UPM participants for more than 25 years. Upon binding of bacteria to the surface of an

acoustic microresonator, the mass and viscoelasticity are modified, and thus so are the resonance frequency and quality factor of the resonator. UPM will develop a conventional quartz-crystal microbalance (QCM) working at resonance frequencies below 200MHz and more advanced, higher frequency (2 to 5GHz) shear-mode bulk acoustic wave (BAW) resonators. BAW devices are grown directly on a substrate and may potentially show higher sensitivity. The UPM has already developed BAW devices and has experience in gravimetric devices for pico-mass and biomolecules.

The LCS relies on the disruption of the intrinsic molecular ordering of the liquid crystal (LC) by the bacteria to be detected. The ordering is typically governed by boundary conditions in the LC cell, which consists of a 1.5-100μm thick layer of LC sandwiched between parallel flat substrates. The ordered structure of the LCS is characterised by macroscopic anisotropies such as birefringence. Any disruption of the order, caused by a microorganism bound to the confinement surface, will lead to a change in birefringence and can be detected optically between crossed polarisers. The viscosity of the LC means that any disruption of the alignment will extend over some tens of μm and thus will get hugely amplified. The relationship between the surface conditioning and the LC order, and thus the signal amplification, has in other contexts been thoroughly studied by the UPM over the last decade although never in the context of bio-detection.

In industrial production, high frequency QCMs have the disadvantage of a higher unit price, compared to the thin film based BAW resonators; however the low frequency detection electronics of QCMs is significantly simpler guaranteeing a final working product. In the case of the LCS, either direct visual or basic optical detection will be exceedingly cheap and easy. The MRS and LCS share the principal design of the microfluidic system, developed by Jonsman Innovation (JINN), and will share the aptamers and the aptamer-binding protocol with the EVA-sensor of DDX.

A number of key development steps and the process of adaptation to industrial manufacturing will be performed or overseen by JINN, a process that the JINN has already undertaken in other contexts.

At the onset of the project a detailed testing plan was elaborated, but given the delay in the manufacturing of specific aptamers, and the subsequent delay in the in-house testing of the sensors, this plan has not been fully executed.

4.1c. Main S&T Results

In the following the main S&T results/foregrounds generated during the execution of the RaptaDiag project will be highlighted. The highlights will be grouped according to the different Work Packages.

Work Package 1: Development and Adaptation of Aptamers

The aim of this WP1 was to develop high affinity specific aptamers against *Neisseria meningitides* and *Streptococus pneumonia* adapted to be used as receptors in the EVA-Biosensor and new low cost sensors (MRS/LCS). This work package was to be carried out essentially by BAPT, but as a consequence of the bankruptcy of this partner, it was completed by the UPM. In the adaptation of the aptamers to the EVA-biosensor BAPT/UPM worked in close collaboration with DDX, and in the adaptation of the aptamers to the Low Cost sensors was facilitated by the transfer of personnel and skills from BAPT to UPM.

Functionalization Protocol

The first main achievement in this WP was the development of a unified activation protocol for the two low cost sensors. This protocol showed to be highly efficient both for antibodies and for aptamers.

The surface activation protocol for the low cost sensors was from the beginning intended to be low cost, compatible with future high throughput production. The two sensors have two very different requirements to the functionalization: micro-resonating sensors (MRS) requires the target species to be tightly bonded to the surface in order to be moved with it, while liquid crystal sensors (LCS) on the other hand requires a surface structure which is, if not aligning in itself then at least, non-disruptive in terms of the alignment of the LC. We have developed two very similar protocols with small differences in order to adapt each of them to such requirements.

These protocols consist of surface functionalization through APTES silanization and covalent attachment of streptavidin through its NH_2. After that, the receptor molecule modified with biotin tag is bound to streptavidin to ensure that its target binding site will be properly oriented.

The scheme of functionalized surface is shown in Figure 2. The BSA molecules are replaced by dsDNA in the case of LCS devices, since the BSA caused misalignment of the LC cells, leading to a high number of false positives.

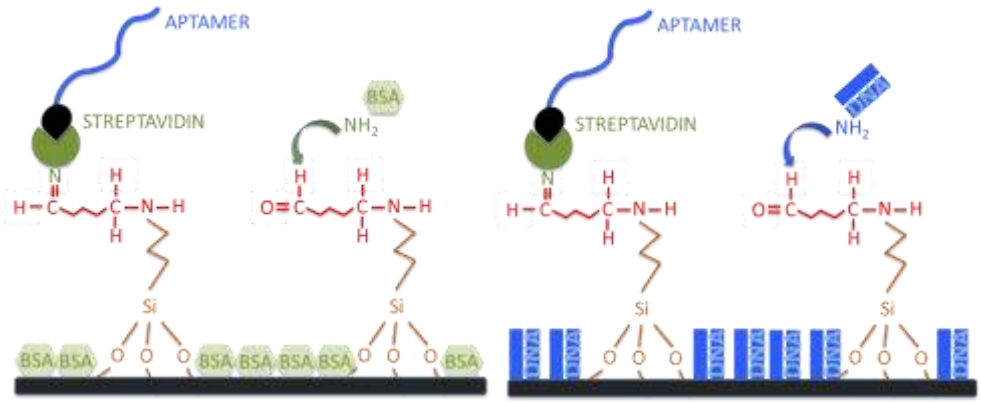


Figure 2: Scheme of surface functionalization for MRS and LCS

The testing of the functionalization protocol was entirely positive selective (figure 3).

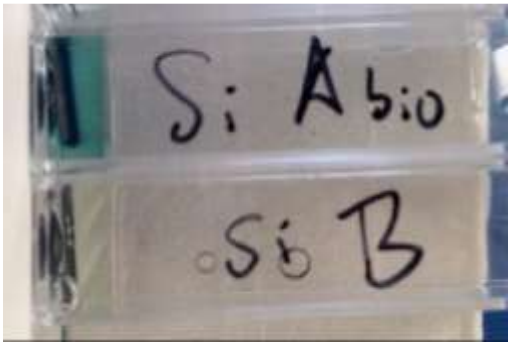


Figure 3: Glasses coated with SiO2 were functionalized with streptavidin. Biotin tagged aptamer (A) and untagged aptamer (B), were used for confirming specific and detecting non-specific surface binding. The complementary aptamer strand and a Horseradish Peroxidase/ABTS assay was used for the reporting.

SELEX against Streptococcus pneumoniae

SELEX rounds against **whole cell** *S. pneumoniae* by traditional "unmodified" SELEX method have been performed. The polyclonal populations obtained were tested with negative results.

SELEX against purified protein is in many cases a better way to achieve specific aptamers and to avoid unwanted non-specific interactions with any molecule on the membrane, cell-wall or capsule in the microorganisms. Thus WP1 included purification of **recombinant proteins** specific to *S. pneumoniae* and *N. meningitidis* surfaces.

We have performed SELEX against choline-binding proteins **LytC** and **Pce** specific from the surface of *S. pneumoniae*, but with no positive results. These are proteins with negative net charge and the only way to isolate them is by using positively charged DEAE sepharose gels. These gels also bind aptamers because of the aptamers negative charge resulting in a non-specific selection.

New SELEX Method with modified nucleotides

In order to improve the chances of a positive outcome for the SELEX against the rest of the proteins, a new, more costly and complicated SELEX process, developed by Somalogic Inc. ["Aptamer-Based Multiplexed Proteomic Technology for Biomarker Discovery", PLoS ONE, e15004, 5(12) 2010] with modified nucleotides was implemented.

Our experience in SELEX tells us that unmodified DNA aptamers have a limited number of chemical groups resulting in less able combinations to bind a target. The affinity of a native aptamer is directed by the negative charge of the phosphate backbone or by the hydrophobicity caused by different nucleotide sequences. The Somalogic method involves pyrimidines modified at position 5 with different aminoacids. In our case, tryptaminocarbonyl was chosen as aminoacid group for dUTP modification (Figure 4). This indole group will allow for additional, non-ionic, chemical interactions between the aptamer and the target, improving their specific recognition. Recognition based on ionic interactions is particularly difficult with gram positive bacteria in which the negatively charged peptidoglycans will tend to repel the likewise negatively charged phosphate backbone of the aptamer. The same applies to the negatively charged proteins.

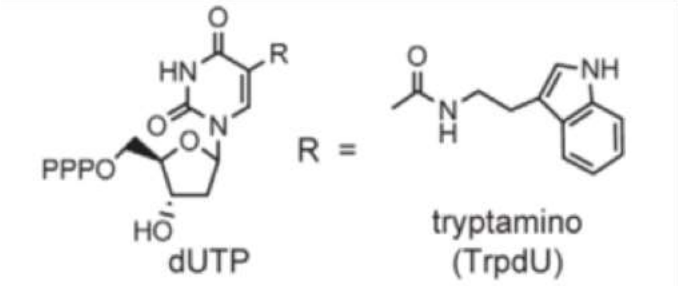


Figure 4: Deoxyuridine was modified by tryptamino addition at position 5

This Somalogic method is quite different from the conventional SELEX, modifying dUTP with a tryptamino group, and having to amplify the DNA using KOD polymerase. The consequence is a more time consuming SELEX steps to obtain single strand and higher costs, but the aptamer binding to the target is similar to antibody binding generating a lot higher affinity.

The **New SELEX method** was applied to **LytA** and **pavA** recombinant proteins specific of *S. pneumoniae* and the recombinant proteins **NadA, fhbp** and **Gna2132** specific of *N.meningitidis*. We did our own protocol with incubation time, blocking reagents, washes and new extension techniques, improving and adapting the Somalogic protocol.

After SELEX against LytA (*N*-acetylmuramoyl-l-alanine amidase), the obtained affinity was not sufficiently high for cloning and sequencing.

Selex against PavA protein.

The denatured and native forms of pavA (fibronectin-binding protein) initially provided by subcontractors group from CIB were no adequate for SELEX because of its insolubility and low expression so we decided on a commercial supplier (Biologics Corp Inc. http://www.biologicscorp.com/) that was able to guarantee the solubility of pavA HIS-tag.

After eight rounds of the modified SELEX two monoclonal aptamers with significant affinity was generated PA08-10 and PA08-19 Figure 5.

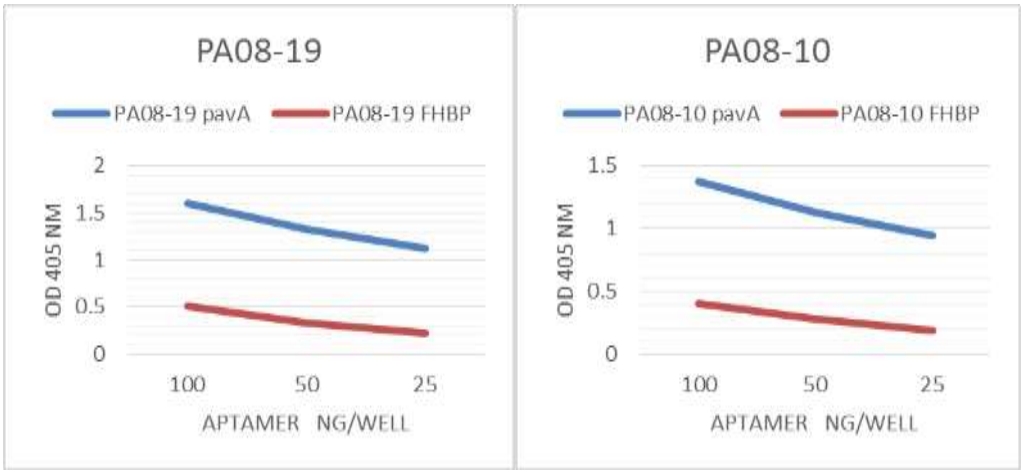


Figure 5: ELONA results for PA08-10 and PA08-19 shows high affinity of monoclonal for the target protein pavA and low affinity for other nonspecific protein FHBP

SELEX against recombinant proteins from *Neisseria meningitidis*

The group of Dr. Ferreirós at University of Santiago de Compostela (USC), which specializes in meningococcus, suggested three proteins specific from the surface of *N. meningitidis* to be used as SELEX targets:

FHBP: Factor H binding protein. http://www.uniprot.org/uniprot/A1E5L5

NadA: QuinolinateSynthetase. http://www.uniprot.org/uniprot/C9X2L6

Gna2132 or Nhba: Heparin Binding Protein. http://www.uniprot.org/uniprot/B9VXA7

The procedure we followed was the same for all the targets and is based on the conditions probed on PavA protein.

Polyclonal SELEX results were obtained for the three recombinant proteins. The fifth generation SELEX for neither of the proteins was improved, although two eights SELEX generations were generated (08 and 08b). 25 clones were obtained and sequenced from the mix of NA05 and NA08 (polyclonal populations from NadA SELEX), 25 from FH05 (from FHBP SELEX) and 25 from GN05 (from Gnd2132). Finally, **22 unique sequences from NA05 and NA08 populations**, **12 sequences from the FH05-population** have been produced, and for

Gna2132 11 sequences. We decided to produce and test 7 from NA05 and NA08, 5 from FH05 and 5 from GN05.

ELONA test of monoclonal aptamers obtained from FH05 and GN05 were negative. However, two monoclonal aptamers from NA05 reached a positive result: NA05-1 and NA05-18 (Figure 6).

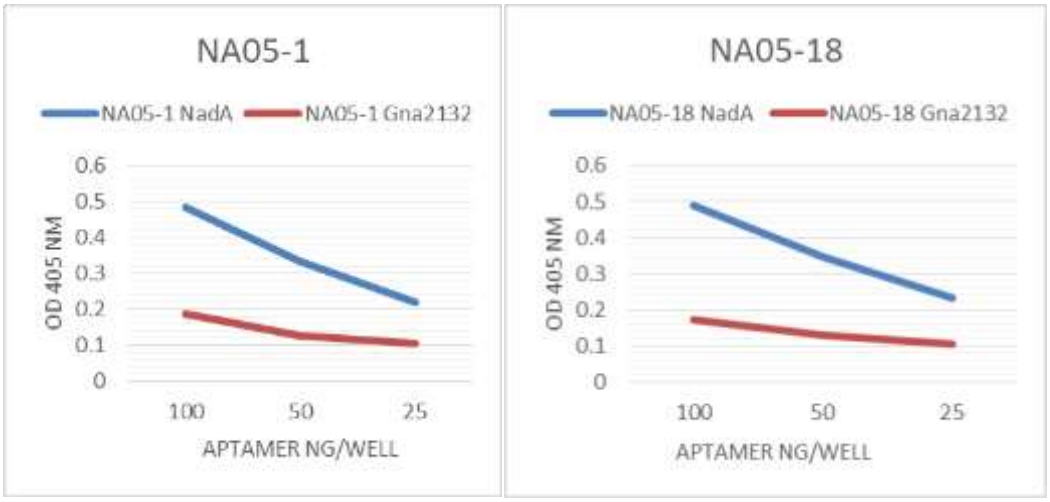


Figure 6: ELONA results for NA05-1 and NA05-18 shows high affinity of monoclonal for the target protein NadA and low for other nonspecific protein Gna2132

The results we have achieved in WP1 are quite positive: we have developed a functionalization method to bind aptamers or antibodies to the surface of both kind of sensors. We have adapted a new SELEX method and thereby avoid that the negative charge of aptamers drives the selection. Finally, we have achieved our goal obtaining monoclonal aptamers specific of proteins of bacteria surface (*pneumococcus* and *meningococcus*) and with a high, albeit not determined, affinity. Next step will be to calculate the dissociation constant (Kd). The results obtained in ELONA have been replicated in the Microresonating Sensor.

Work Package 2 Adaptation of Eva-Chip to Aptamers & EVA-Reader Demonstrator

WP 2 covers two main areas that were investigated jointly with UPM and JINN, namely translating the EVA-Technology into a portable EVA-Reader demonstrator for the point-of-care environment to deliver robust, fast and reliable results within 10min and to develop new coating protocols and processes suited for the use of aptamers in different assay configurations. The different coating protocols were adapted, optimized and tested for the detection of proteins and bacteria as targets –with a special focus on the latter.

EVA-Reader for Point-of-Care & Development of a Demonstrator

As basis for the test with aptamers, a portable RaptaDiag EVA-Reader has been developed within RaptaDiag for point-of-care settings. The focus here was on usability, ease-of-use and robustness that had to be fulfilled while still warranting a high quality of the results. The demonstrators realized (Figure 7) were used to develop and investigate the novel aptamer as detection molecules but also to measure, assess and demonstrate the specific features advantageous for point-of-care.

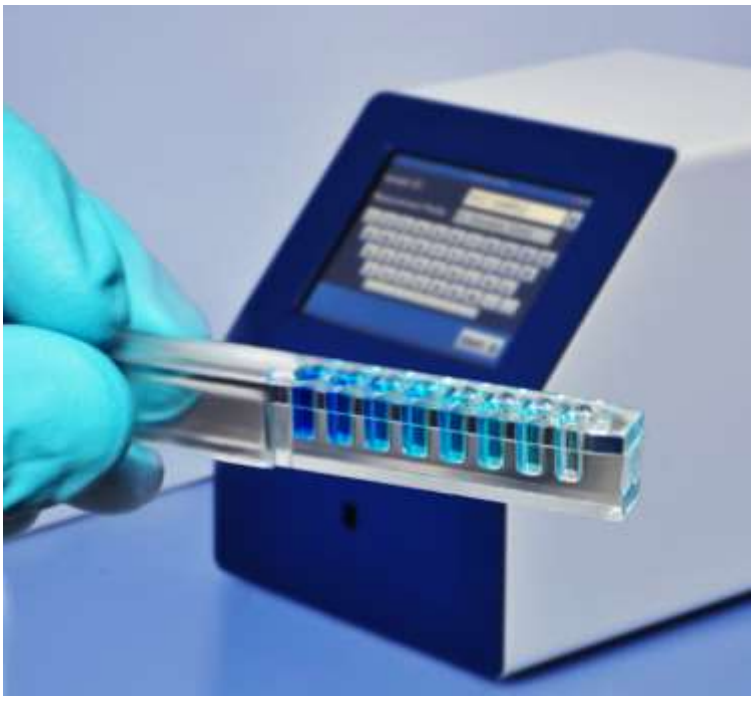


Figure 7: Picture of the core elements of the realized RaptaDiag EVA-Reader: EVA-Reader (background) and EVA-Chips (foreground)

The portable demonstrator developed is based on the EVA-Technology that exploits the phenomenon of evanescent fields as illustrated in the scheme below (Figure 8). Due to the

limited penetration depth, only the first 200 nm at the bottom of each well are illuminated and only the fluorescence signal within this surface confined area contributes to the signal that is measured by the detection module. In combination with a time-dependant readout (measuring the increase of the fluorescence signal for 10min), this lead to a system that can detect the concentration of biomolecules such as aptamers in a very robust manner well suited for point-of-care applications.

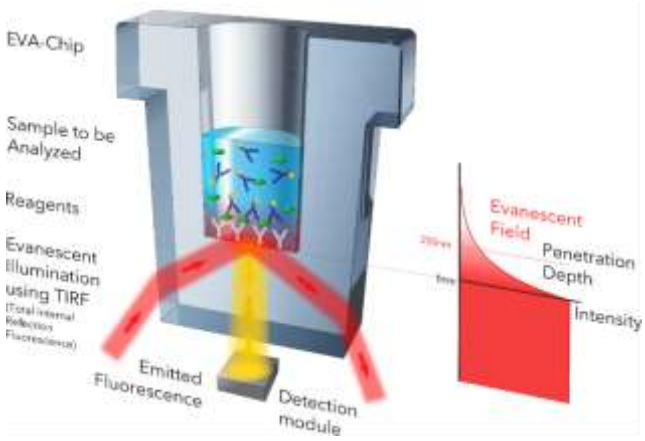


Figure 8: EVA Technology (evanescent fluorescence excitation)

The limited and well controlled penetration depth is also an important element to achieve robust results in hectic point-of-care setting where pipetting highly precise sample volumes is typically not possible. Therefore is required that that the EVA-Reader is very tolerant with respect to any changes in the sample volume. Figure 9 shows the volume dependence for a reference assay (beta HCG) where the wells were filled with different volumes from 10µl (less than a drop) to 90µl (well completely filled). It can be seen that for a sample volume of more than 20µl –i.e. as soon the bottom surface of the well is completely covered –the measured signal becomes nearly *volume independent.*

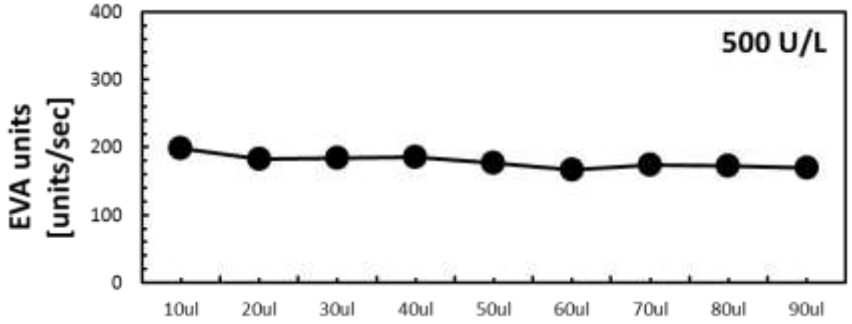


Figure 9: Volume dependence of the EVA-System demonstrator for different concentrations.
➔ No need for pipetting accurate volumes makes the test easy-to-use and robust!

Additional key points that have been developed and implemented in the RaptaDiag demonstrator is the ease of use by developing an *intuitive, self-explanatory user interface* on the touch screen with *minimal user interactions* (Figure 10) and by adapting the assay configuration to a *robust single step assay*: Just add the sample to the *pre-coated disposable aptamer EVA-Chips* (minimal manipulation steps and elimination of any error-prone sample dilution to increase robustness), and load the EVA-Chip into the instrument to obtain the quantitative results within 10min.

Figure 10: EVA-Reader start-up screens

In point-of-care environments a power plug is not swiftly available so that the EVA-Reader demonstrator should be able to run on portable power. The EVA-Reader demonstrator realized can handle voltages from 12-60 VDC and uses very little power (5 W while measuring, 2-3 W in idle mode) which can even be delivered by few AAA batteries (capacity of 2500 mAh each) for some hours. This has been demonstrated and the most practical solution was to use an external rechargeable 12 V battery pack as they can be switched easily to extend the operating time.

Figure 11: Series of injection molded EVA-Chips realized within RaptaDiag

Throughout RaptaDiag the reproducibility of the measurement results was addressed by production of large batches of injection molded EVA-Chip (Figure 11) and by developing suited assembly and calibration processes for the EVA-Reader to obtain identical results from all readers. This crucial step that is often underestimated could be successfully demonstrated using a series of 20 EVA-Readers (see picture in the "impact" section) that have been produced in parallel by DDX.

Coating Protocols for Aptamers

For the use of aptamers, several different coating protocols for different assay formats and targets (proteins and cells) were developed. One of the novel coatings that was developed and optimized within RaptaDiag for *L. pneumophila* was direct immobilization of whole bacteria on the polystyrene surface of the EVA-Chip. The detection of a constant amount of bacteria is achieved by addition of increasing concentrations of the *L. pneumophila*-specific Cy5 labelled aptamer Leg01 (Figure 12). The EVA signal increases proportional to increasing aptamer concentrations. A plateau is reached at a concentration of 1.3 µg/ml. The best aptamer concentration was used later for a cell titration experiment in which increasing amount of cells were immobilized on the chip surface. This assay format delivered a detection limit of 56,000 *L. pneumophila* cells/ml (1700 cells/well).

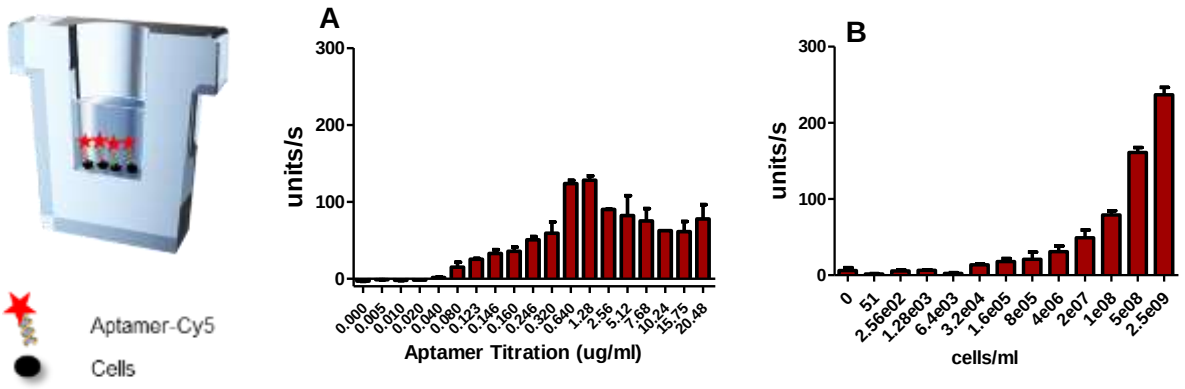


Figure 12: Direct cell immobilization Assay for the detection of L. pneumophila. (A) Aptamer titration experiment; (B) Cell titration experiment

To identify the best suited approach, also different assay formats were investigated. As example an indirect immobilization approach using biotinylated polyclonal Anti-*Legionella pneumophila* antibody which was bound to a NeutrAvidin™ surface for the immobilization of the cells is shown in the Figure 13. The same experiments were performed as for the direct immobilization assays: in the aptamer titration experiment (Figure 13), a plateau was reached at a concentration of 11 µg/ml, which lead to a limit of detection for this assay of 1e08 cells/ml. Compared with the results obtained with a direct immobilization assay, the assay using a polyclonal antibody is clearly less sensitive for this specific target and is an illustrative example for the different methods for aptamer based detection using cells and proteins respectively.

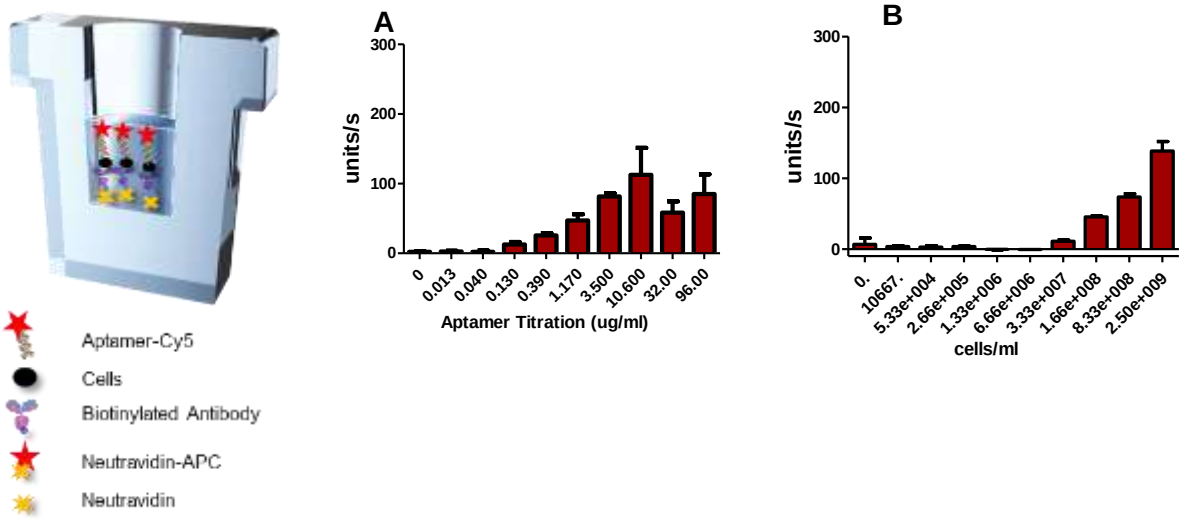


Figure 13: Indirect cell immobilization Assay for the detection of L. pneumophila using the polyclonal antibody. (A) Aptamer titration experiment; (B) Cell titration experiment

Within RaptaDiag fast, sensitive, and specific aptamer based assays for the detection of the pathogenic bacterium *L. pneumophila* have been developed and the novel aptamer protocols could be established for the rapid biosensor systems. Throughout the project different optimized protocols for proteins and cellular targets have been developed and successfully established.

Work Package 3: Low Cost Aptamer Detectors

The objectives of this specific WP was to develop, and validate very low cost aptamer biosensors using aptamers. Three kinds of detectors have been successfully developed. As a low risk sensor, a quartz crystal microbalance (QCM) working at high frequency has been produced. The alternatives, shear-mode bulk acoustic wave (BAW) resonators and liquid crystal detectors have likewise been produced. In all cases, the associated readout electronics has been developed, paying special attention to portability and cost. The results include unprecedented sensible BAW-resonators for detection of pathogens in aqueous solutions, and a patented novel detection system based on lyotropic liquid crystals with a fully portable reading unit which with a bit further maturing of the technology will become a portable, low-cost point-of-care diagnostic system allowing a rapid quantitative diagnosis.

Gravimetric sensors.

The first developed system in the project was a quartz crystal microbalance (QCM) based system consisting in a stable oscillator using a QCM as feedback element, a precision counter, and a laptop computer interface. The main objective was to reduce the final cost maintaining the typical performance of the system. The system was described in detail in deliverable D3.2. For exciting and readout the signal from the resonant crystal an oscillator with automatic gain control (AGC) and a capacitive compensating branch were designed and fabricated. The AGC is implemented for monitoring the losses of the crystal, sensible to changes in liquid viscosity, in addition to the frequency changes. The oscillator showed a good long term stability with a temperature coefficient of frequency of around -30 ppm/K.

The readout system was fabricated with standard logic circuitry and a temperature compensated quartz crystal as reference. A microcontroller allows controlling of the readout process and interfaces with a computer through USB standard interface. The whole system has a resolution better than 1 Hz.

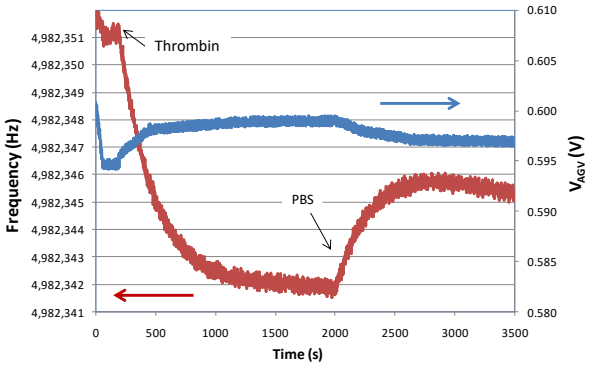


Figure 14: Frequency evolution of the QCM in a thrombin detection process

To test the response of the system we used thrombin detection with TBA-29 aptamers. In Figure 14 a typical detection of thrombin with the QCM system is shown. At this point of the

project the functionalization protocol was not yet well established and non-specific binding dominates the detection process.

In the second stage, high frequency (in the 1.3 GHz range) resonators made of thin films of AlN were developed. The system was described in detail in deliverable D3.4. For this part of the project, a deposition method of thin AlN films with the c-axis of the microcrystals tilted respect the surface normal was optimized. This particular morphology of the AlN film is needed for generating shear resonant modes, which can operate in liquid medium. In Figure 15, a scanning electron microscopy (SEM) image of a cross section of the resonator is shown.

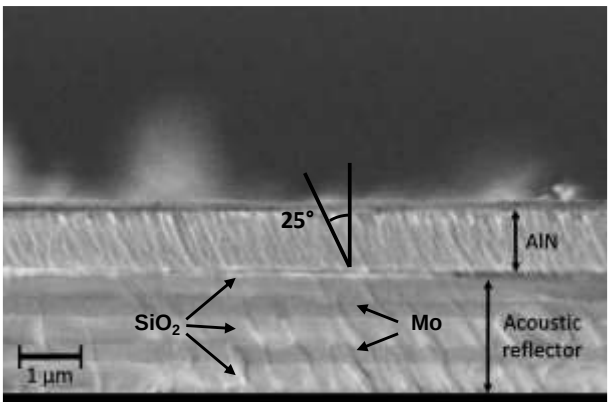
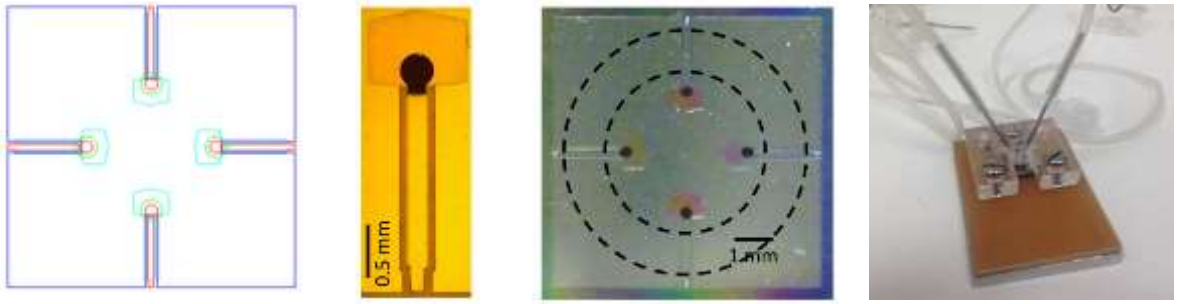


Figure 15: SEM image of a cross section of a typical shear mode AlN resonator

The whole fabrication technology of high frequency detectors was developed and optimized, including the studies of the effects of encapsulation, for which electrical extension is needed. The fluidic holder consists of a PMMA laser machined piece with 1mm diameter needles attached for liquid feeding. The sensors are isolated from the air through a nitrile O-ring of 4 mm in internal diameter and 1mm width. Figure 16 shows the final layout of the devices and the fluidic system for laboratory use.



a) b) c) d)

Figure 16: a) Layout diagram, b) single device, c) chip with four devices of the final design of high frequency sensors, and d) the fluidic system. The dashed lines in c) are the O-ring footprint

Most of the tests made with these sensors were performed by monitoring the resonant frequency evolution of the sensor with time with a network analyser while the different

liquids used for functionalization and detection were fed on to the sensor surface using a peristaltic pump.

The resonant frequency was determined by measuring the frequency for the maximum of the real part of the admittance. The application designed for measuring and fitting was made in LabView® and allows measuring the frequency every 3 seconds with a noise floor from 1 kHz to 4 kHz (typically 2 kHz) depending of the particular device.

Devices were calibrated by depositing on them very thin SiO_2 films of well-known thickness and density (and therefore, mass) and monitoring the frequency shift. The mass sensitivity resulted to be 1800 gr/kHz·cm^2. Taking a value of five times the noise floor as criterion for detection limit, the lower mass that can be measured with the sensors (low detection limit, LOD) is 4.2 pg.

To demonstrate the viability of low cost complete system a simple microstrip based oscillator was designed and prototyped. This first prototype is shown in Figure 17. The design allows a continuous oscillation with an Allan deviation of around $2x10^{-7}$, enough to obtain the desired resolution. A more complex design with better stability probably would allow reducing the LOD with a very low cost circuitry.

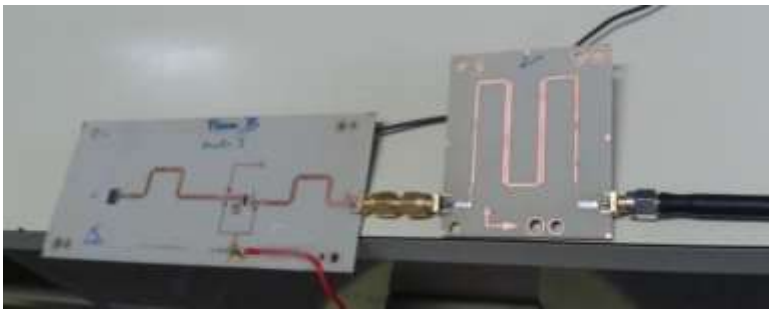


Figure 17: Microstrip oscillator using a high frequency sensor as feedback element

A complete functionalization process was developed for using the sensors as a label free biological detector as described above. This process has two particularities for these devices. First the functionalization surface is made of iridium oxide, which is deposited in the same process that the top electrode eliminating a photolithographic mask level and a SiO_2 deposition process. Second, the generation of the OH^- groups needed for APTES binding is made by a short O_2 plasma immersion, which substitutes the piranha etching which deteriorate the metals atop the device. These modifications make the functionalization quite similar to the traditional one using SiO_2 and piranha etching, being even faster.

To test the sensors, functionalization with TBA-29 aptamer was performed and thrombin was detected. In Figure 18 the response of a typical sensor is shown. Similar response was obtained by functionalizing the surface with antibodies (anti IgG mouse to detect IgG mouse). Also Legionella bacteria detection was performed with antibodies. The detection of the whole

bacteria was not possible, but when the targets were solubilized by sonication detection was possible. The lack of detection of whole organisms could be due to the binding of bacteria to the surface by its lipopolysaccharides which makes the bond very flexible and, together with the in-liquid friction forces, hampers its vibration.

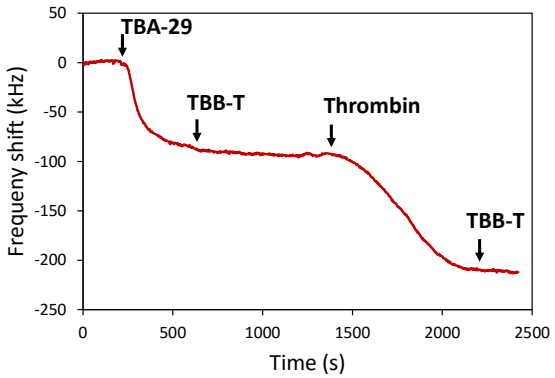


Figure 18: Frequency evolution of the sensor in a thrombin detection process

As conclusions, high frequency gravimetric sensors show a very good sensitivity and very high selectivity, the non-specific binding is very low being the negative detection (test for non-specific binding) very clear and reproducible. This last property is very interesting to be applied in the selection and purification process of aptamers, which is an idea that is now being developed. Unfortunately, whole bacteria cannot be detected with a conventional surface morphology; however, there are alternatives to be tested. Mass fabrication will make the sensors itself very cheap. This, together with the possibility of design arrays for multi target detection, makes this technology very promising for low-cost and high-reliability biosensors.

Volumetric Liquid Crystal based sensors

The most surprising positive results in this project may be assigned to the development of the low cost liquid crystal sensors. While not having reached the matureness of neither the microresonating sensors, nor of course the EVA-sensors, a number of very important milestones have been reached, and the projection of this technology into the future is promising. The initial approach was to combine the results of the two dominating groups in the field of LC biosensors, by using water based *lyotropic* liquid crystals, as employed by the Liquid Crystal Institute, Kent State University, with functionalised alignment surfaces as the Abbott lab, University of Wisconsin. Both of these technologies are based on the alignment disruption caused by the presence of microscopic corpuscles in the LC matrix. When looking at these cells aligned with and situated between crossed polarisers, the misaligned areas become visible as shining dots or areas.

Alignment and materials

The first challenge was to align the lyotropic on a surface compatible with functionalisation. The initial alignment was done using polyimide and the most conventional lyotropic liquid crystal (LLC) Cromolyn (Figure 19 and Figure 20).

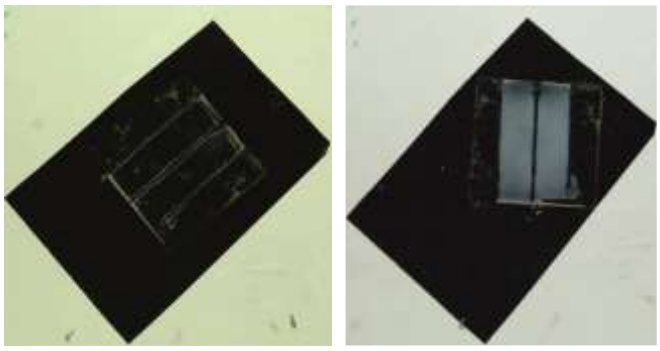


Figure 19: Example of the alignment of Cromolyn in polyimide

The alignment disrupting effect caused by 4µm silica beads in the LC was visible, albeit with a limited amplification factor of approximately 10x.

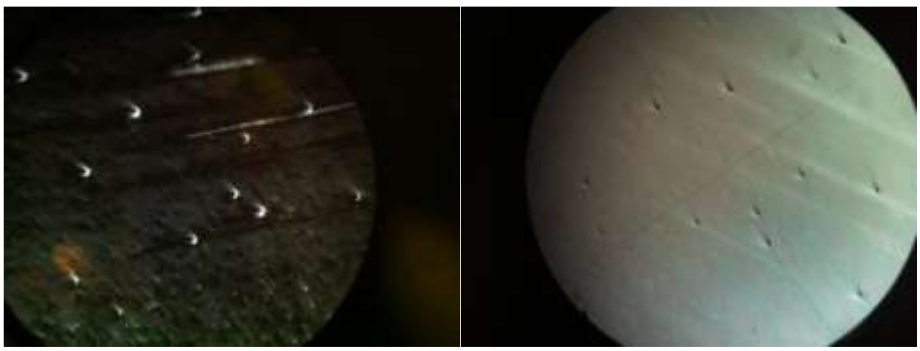


Figure 20: A sample with the presence of 4 µm silica beads aligned parallel and at 45° with respect to a set of crossed polarisers. The amplified signal exceeds 50 µm in size, which is on the limit of what can be appreciated with the naked eye

However, since the consortium was targeting one common activation protocol for both of the low cost sensors, it was decided to target obliquely evaporated inorganic SiO_2 alignment, which had never been used with lyotropic liquid crystals. Furthermore, this surface also showed less negative capillarity making it easier to fill the cell with LLC.

It was also decided to swap Cromolyn for a less viscous LLC *Sunset Yellow (SSY)*, which has furthermore shows an LLC behavior in a wider temperature and concentration range, making it a more flexible LLC to work with.

An SiO_2 evaporation angle of 60° was found suitable for the SSY although it became clear that only a very limited amplification was the result employing this combination of SSY and SiO_2 (Figure 21)

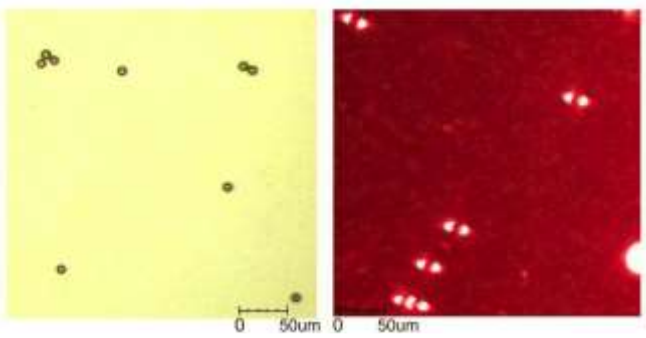


Figure 21: Cell as seen under the microscope between crossed polarizers at 0º (left) and at 45º (right). Very limited amplification of the silicon beads (factor 3-4) is achieved

Dynamic flow

The limited response incurred above was more than mediated by applying a slight flow to the cell, which resulted in an unprecedented signal magnification (Figure 22)

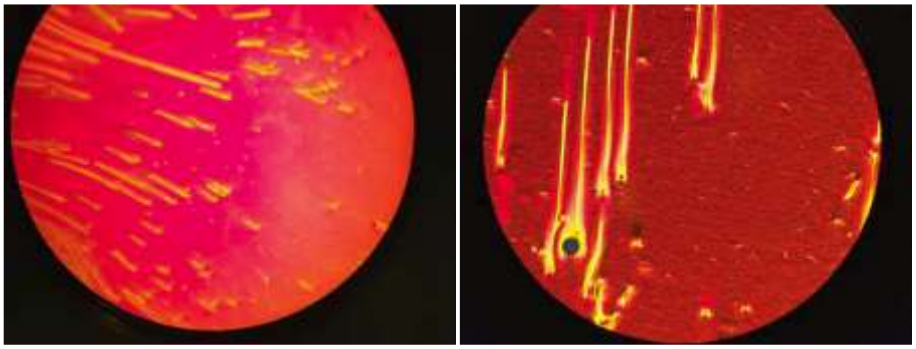


Figure 22: Cell as seen under the microscope between crossed polarizers. The misalignment effect in the liquid crystal is observed while flowing. The amplification factor depends on the flow velocity, but a factor of 50 was easily achieved, whereby the targets became visible to the naked eye.

This result was transferred to a patent (ES2499790 (B2), WO2015193525 (A1)), which was finally grated in July 2015. The flow applied to the cells caused a long tail of misaligned liquid crystal to become visible downstream to perturbation immobilized on the surface of the cell. Much like a rock in a steadily flowing stream or a boat generating a wake on a quiet lake. The flow in these cells was generated by capillary effect when filling the cell.

Target detection

While the above results were promising the problem was to transfer the results generated in a LLC cell with silica spacers directly on an SiO_2 surface to cell with at least one functionalized surface, without losing the alignment ability and without causing too much nonspecific binding of any targets. Significant effort was invested in establishing the details of the functionalization protocol, as presented in WP1, and the specific washing and loading protocols. These protocols meant that a fluidic system, similar to that of the microresonators had to be developed. The main problem to overcome with such a system was that the gap dimension of the LLC cell could not exceed 60μm, which meant an important backpressure, and consequently the need for a well sealing microfluidic unit.

A large amount of different flow cells based on PMMA (Figure 23) was designed, and sealed using epoxy.

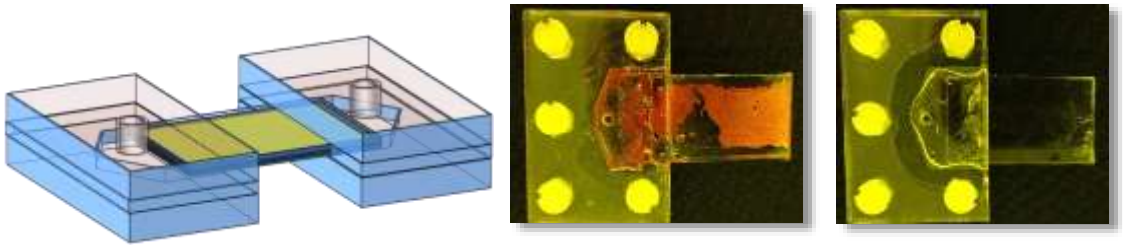


Figure 23: One of many prototype flow cells for the activation, passivation and evaluation of the LC sensors.

In order to assess the potential as assay, cells were functionalized with legionella (positive) and mouse (negative) antibodies, and then loaded with a dilute solution of fixed legionella. After incubation, the cells were washed with SSY, and left 10 minutes without applying any additional external pressure. A difference in the cells was generally detected (Figure 24). A thorough study of detection and noise levels is still pending

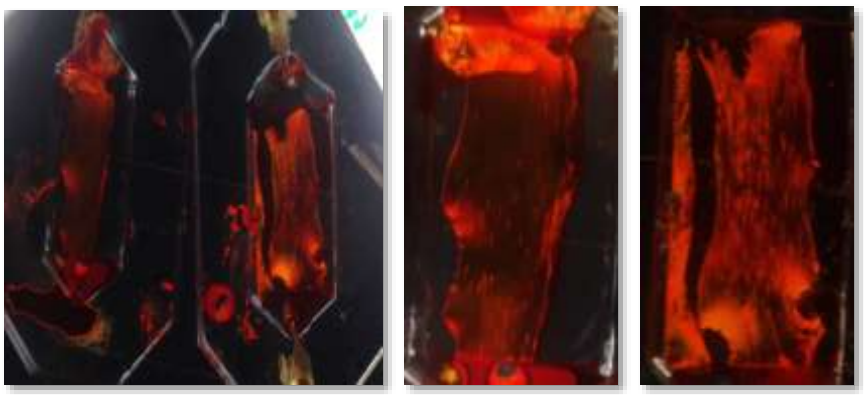


Figure 24: An example of a blank and a positive sample. Clearly the positive sample shows a higher macroscopic transmittance, and a lot less points of alignment interruptions.

LC reader

It became clear that in the case of the LC sensor a recording unit providing unbiased documentation of the measurements was needed. Thus the LC reader, Figure 25, was built based on a commercial slide scanner, with the addition of the necessary crossed polarisers

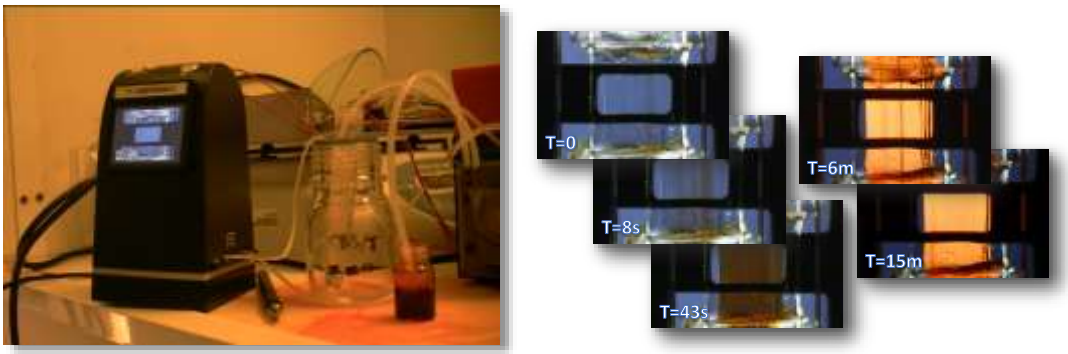


Figure 25: The final LC reader in the process of testing and resulting images. A full video of the data capture of a test run is available at http://1drv.ms/1S34imZ

Work Package 4: Microfluidics, Encapsulation and Preparation for Manufacture

WP4 relates to two primary activities:

1. **Microfluidics**: Development of the sample handling microfluidic parts for both the microresonator sensor and the liquid crystal sensor.
2. **Industrialization**: The streamlining and control needed for industrialisation of the technology.

Industrialization

In this work a number of industrial aspects of the low-cost sensor was analysed and evaluated.

1. **Evaluation of manufacturing procedures**
    a. The suitability of different manufacturing procedures and processes was evaluated in this task.
    b. The evaluation addressed the microfluidic assembly, the liquid crystal section and the MEMS microresonator parts. Focus was on manufacturability, low-cost and simplicity.
2. **Industrialisation of detection system**
    a. Planning, maturing and adapting the developed detection systems for industrialisation.
    b. This task was focused on the streamlining and simplification needed to industrialize the developed detection systems. Tolerance sequences was analysed, evaluated and planned with the aim of conforming to a six-sigma production methodology. A top-level Failure Mode and Effects Analysis (FMEA) was made, in which critical process steps were identified and possible alternatives identified and evaluated.
3. **Identification and screening of foundries and sub-suppliers**
    a. Foundries and sub-suppliers for the microresonator, the interconnect PCB and the microfluidics sample handling parts, were identified, in many cases also inspected, and evaluated on their ability to mass produce a non-traditional hybrid sensor device (Figure 26).

Figure 26: Automated production of injection moulded microfluidic cartridges

Microfluidics

The fluidic cell and chip encapsulation for both, the liquid crystal and microresonator sensors, was develop and optimized (Figure 27, Figure 28, Figure 29).

Key focus areas were reliable sample handling and bubble-free filling of the read-out cells, practical usefulness of the developed devices, and ease of integrating the hybrid materials; silicon chip and the polymer microfluidic cartridge, liquid crystals and the polymer microfluidic cartridge.

Microfluidics example: The fluidic cell for the liquid crystal sensor cell

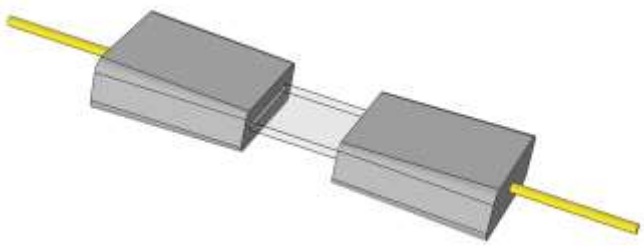


Figure 27: The Solidworks CAD design of the glass fluidic cell (clear in the center), two PEEK tubes for inlet and outlet of the liquid sample (yellow), and moulded silicone connectors (grey)

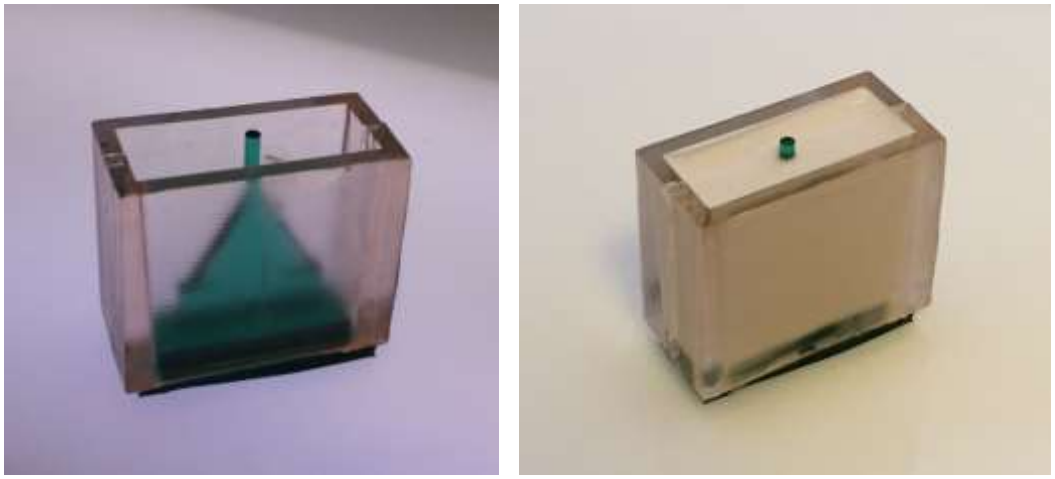


Figure 28: Left, the assembled 3-part mould for the silicone connectors. Right, the silicone mould filled with RTV silicone.

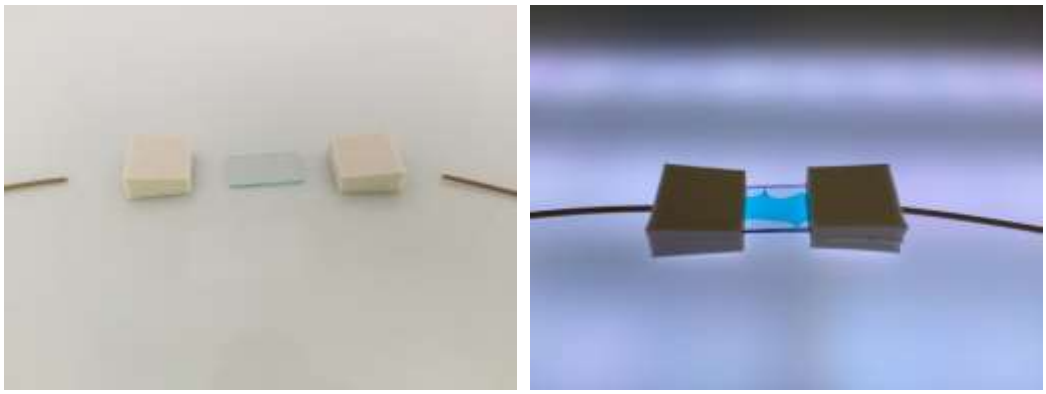


Figure 29: Left, the glass cell, two silicone adapters and two PEEK tubes prior to assembly. Right, the parts after assembly, and the cell filled with blue test liquid.

4.1d Impact

The potential impact of this project is well beyond its original scope. The procedures, methods and protocols developed here can be applied, as a whole or partially, to a significant number of fields and situations, not only in the healthcare area, but in several other subjects that make the potential products to have high economic and wide societal relevance.

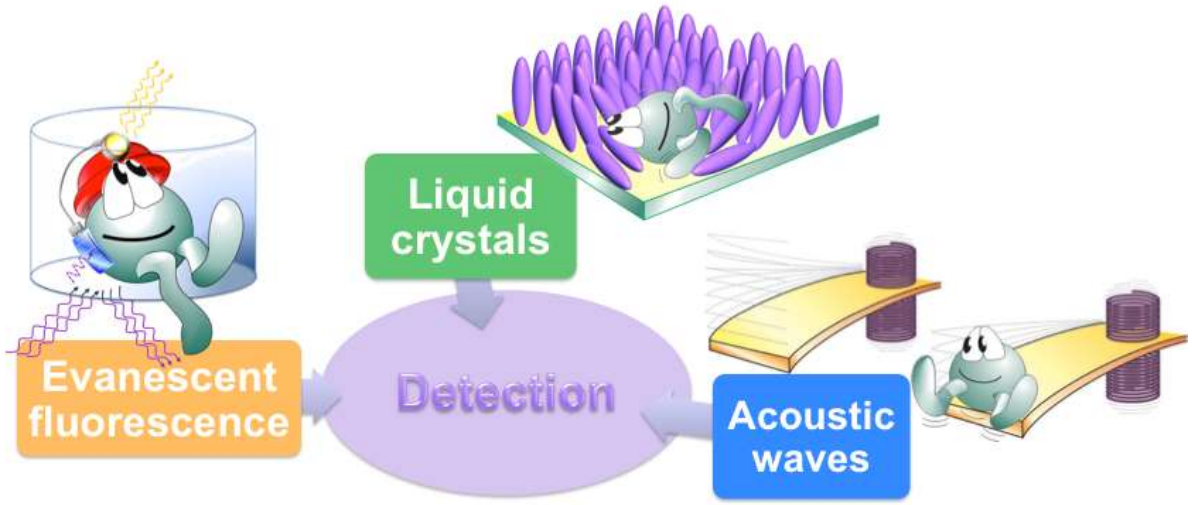


Figure 30. Three different approaches to bacterial meningitis detection having different cost, reliability, sensitivity, and availability.

The key for this achievement has been the early decision of developing three parallel detection methods for the bacterial meningitis (BM) diagnostic test kits (Figure 30). These three methods were not intended to be competitors to each other: actually, the three approaches had in principle a significantly different degree of development, their expected performance and cost were quite dissimilar, and their application sites are unrelated as well. The above notwithstanding, it is important to point out that the three technologies feature a common core, i.e. the use of tailored aptamers for selective detection of the target pathogens. A good deal of the realized work benefits the three branches: aptamer generation and surface functionalization. Just the detection procedure is different in every case.

EVA Sensor: This technology is perfectly mature, and was already commercial in a different version prior to the project start. It is a technology well suited for a standard laboratory environment, to perform routine analyses with reliability and easy handling. The progress undergone during the progress has been obviously limited, since the technology was already demonstrated using antibodies. Yet the use of aptamers could open new possibilities to its current performance.

***Acoustic wave sensors*: The progress experienced by this technology over the project has been outstanding. The technology (**

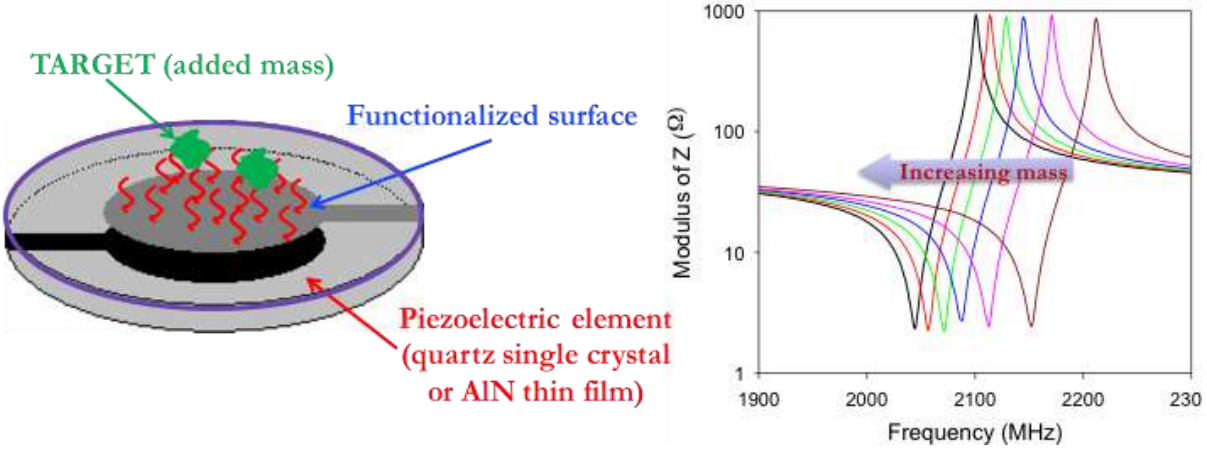


Figure 31), originally designed on quartz crystal microbalances (QCM) in the MHz range, has evolved to thin-film *bulk acoustic wave* (BAW) devices, working in the GHz range and having an unprecedented sensitivity three orders of magnitude above QCMs. This leads to a detection limit nearly 1pg, i.e., the weight of a single bacterium.

Although this limit has not been reached yet, detection of minute amounts of pathogens (4pg) *in liquid* is documented, making this technology an extremely useful tool for early detection of the disease thus reducing the risk of infection and speeding up the application of the suitable therapy. This sensitivity is useful in many other situations, either different infections in healthcare or other conditions where detection of harmful pathogens is required.

An added value of this technique derives from the minute size of the AlN sensors. These devices feature dimensions in the hundreds of micrometers range. This paves the way to the design of more complex units having several, even dozens of different sensors integrated in the same lab-on-chip and controlled by a common microfluidic system.

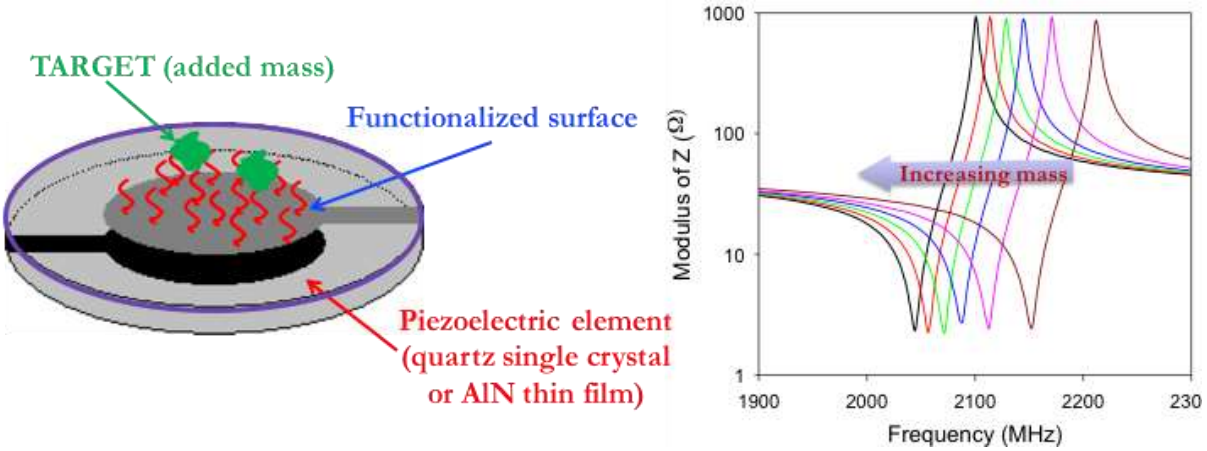


Figure 31. A sketch of a gravimetric sensor and frequency response of the resonance

Lyotropic liquid crystals: Liquid crystal technology was proposed having in mind that it was the riskiest, and that the chances of obtaining relevant results were not high. Indeed this was the situation along the first half of the project. These circumstances dramatically changed in the second half, when a new strategy based on *lyotropic* liquid crystals (LCLs) was adopted. These are quite common materials, present in many instances of biological materials such as cell membranes, liposomes, soaps, and micelles. Nevertheless, except for a few specific applications, LCLs are not technologically relevant. Consequently, very few procedures and manufacturing protocols can be found in the literature, making the work with LCLs to start literally from scratch. Yet there was a strong reason for the team to adopt these materials: opposite to regular liquid crystals, LCLs are soluble in water and hydrophilic media, i.e., the media where target bacteria are usually found.

The breakthrough in this technology was achieved when the amplification of the optical signal was multiplied by a factor of 100× or more, thanks to the observation of the samples with *flowing* LCL rather than static measurements. Such dynamic detection allows the observation of the ***wakes*** left by the non-aligned LCL flow that remain for a few minutes in standard conditions (Figure 32).

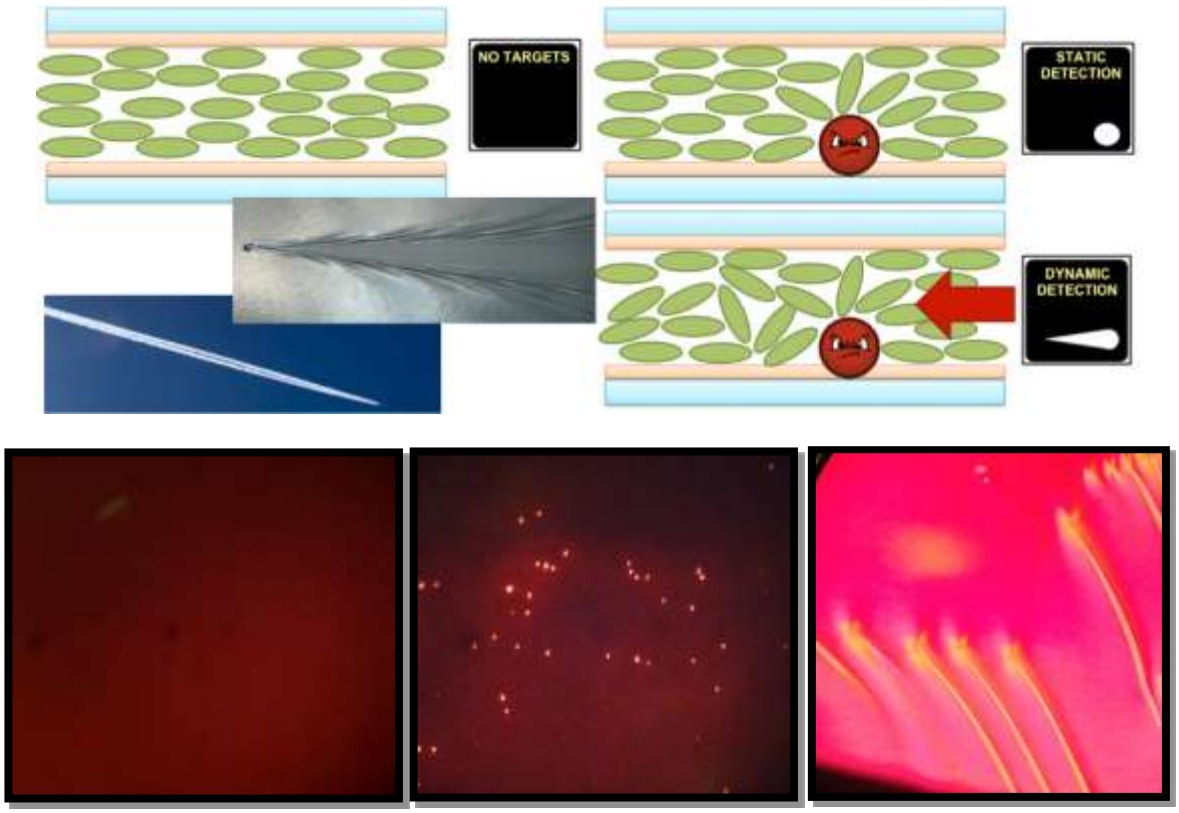


Figure 32. (above) Static detection has a limited amplifying power, restricted to the surroundings of the microorganism. Dynamic detection shows up wakes/trails produced by turbulent flow. As in ships or planes (middle left), wakes and trails are hundreds of times larger than the vehicle itself. (Bottom) LCL

samples with no target microorganisms (left), targets in static detection (middle) and wakes in dynamic detection (right)

Static detection of microorganisms with LCLs had been described more than 10 years ago, the procedure requiring the mandatory use of a microscope for the bright spot were just about 5× the size of the target. Dynamic detection, on the other hand, relies on wakes 100-500× the size of the target, making the detection visible with naked eye.

This is not a mere increase of amplification. Indeed, making LCL test cells visible to naked eye converts a laboratory test into a *field test*. LCL tests are unplugged, light weight, portable, and cheap, making them ideal to be handled out of the laboratory by non-trained personnel –a quite different scenario from the two previous tests. Actually LCLs cannot compete with the accuracy and reliability of other laboratory methods: it is only semi-quantitative, and prone to false positives. Yet it can demonstrate to be an invaluable tool for primary care and large screenings, leaving more detailed tests to the backup laboratory.

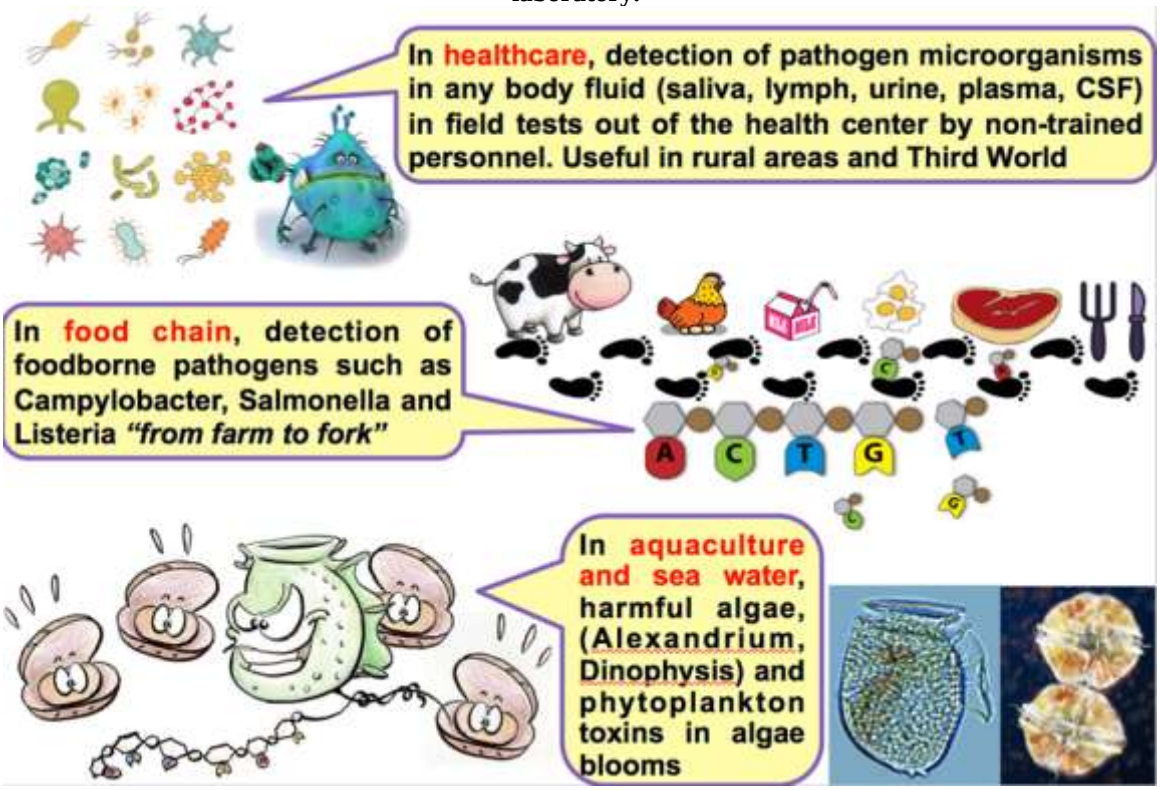


Figure 33 shows proposals of uses in several areas.

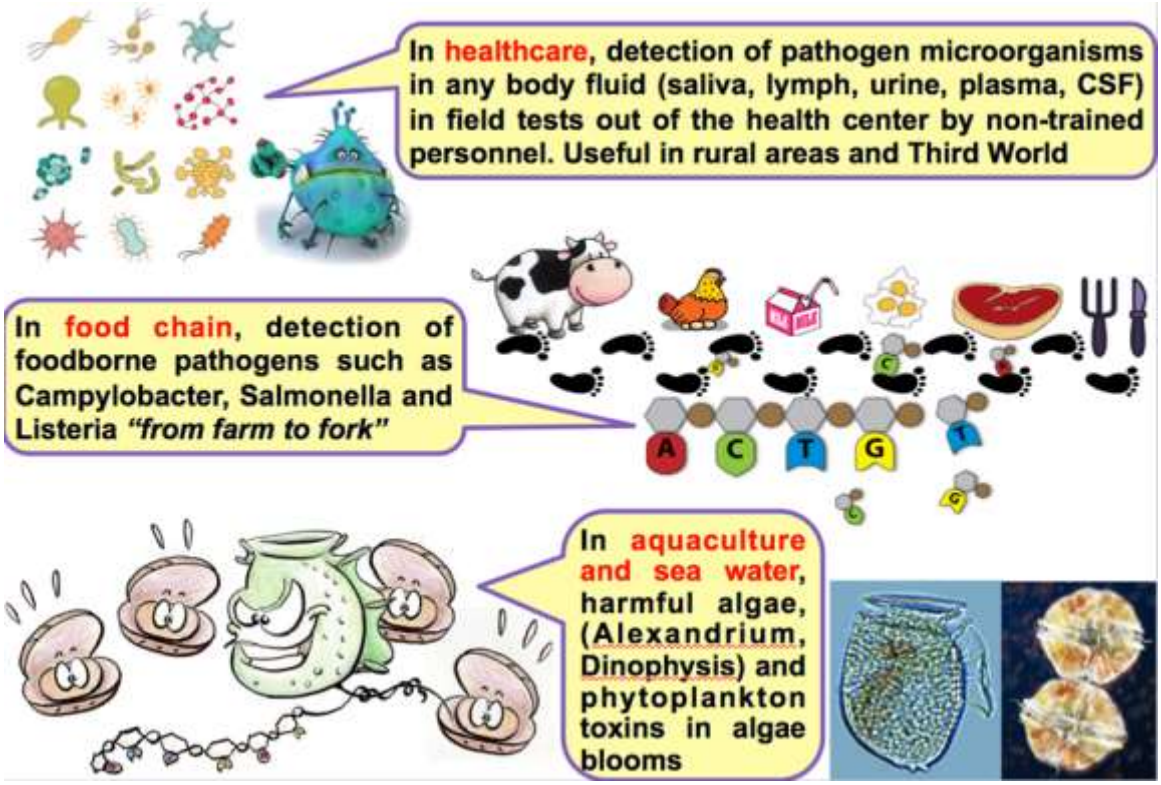


Figure 33. Several proposed applications of field tests like those evolving from LCL sensors

Summarizing, the parallel development of three different detection methods for the same target microorganisms has proved to be extremely fruitful from the technical point of view, yielding potentially commercial products in all cases, having diverse scope and applications. Some of the technologies may eventually find applications well beyond the goal of the project, even beyond the healthcare area. The dynamic detection method has been patented.

The project has had scientific impact as well. Several publications and conferences have derived from the research work, especially the advances in acoustic sensors and LCL cells. Three post graduate students (Mario de Miguel, Teona Mirea, Eva Otón) have focused their PhD work on this subject obtaining achievements partially related to the project objectives. Further research lines on the same subject are currently under development.

For Davos Diagnostics the RaptaDiag project brought a completely novel family of biomolecules, namely the aptamer to the portfolio. The EVA-Reader demonstrator realized has also been tested in different laboratories of opinion leaders in Europe with a positive feedback (Figure 34). As result a first series of 20 EVA-Reader instruments could be sold already during the project duration and the demand for instruments and disposable EVA-Chips is growing after DDX has achieved the status of an ISO 13485 certified company. DDX is currently on the way to translate the EVA-Reader Demonstrator and EVA-consumables into CE-Marked in-vitro diagnostic (IVD) products produced in Europe leading to the need for personnel at DDX (DDX seeks to hire 3 persons in 2016) and to additional marketing personnel for the European distributors of DDX products.

Figure 34: Impact & Testimonials of users of the Portable EVA-Reader Demonstrator

Dissemination

The results of the project has resulted in a 27 peer reviewed articles and publications and in 21 conference presentations.

Journals

1 *"Aligning lyotropic liquid crystals with silicon oxides" E. Otón, J.M. Escolano, X. Quintana, J. M. Otón, M. A. Geday Liquid Crystals 42(8) Taylor & Francis online 2015 1069-1075 10.1080/02678292.2015.1024767*

2 *"Assessment of the acoustic shear velocity in SiO2 and Mo for acoustic reflectors" M. DeMiguel-Ramos, T. Mirea, J. Olivares, M. Clement, J. Sangrador, E. Iborra Ultrasonics 62 Elsevier 2015 195-199 10.1016/j.ultras.2015.05.017*

3 *"Optimized tilted c-axis AlN films for improved operation of shear mode resonators" M. DeMiguel-Ramos, T. Mirea, M. Clement, J. Olivares, J. Sangrador, E. Iborra Thin Solid Films 590 Elsevier 2015 219-223 10.1016/j.tsf.2015.08.010*

4 *"Influence of liquid properties on the performance of S0-mode Lambwave sensors: A theoretical analysis" T. Mirea, V. Yantchev Sensors and Actuators B: Chemical 208 Elsevier 2015 212-219 10.1016/j.snb.2014.11.026*

5 *"Effects of biologically compatible buffers on the electrical response of gravimetric sensors operating at GHz frequencies" M. DeMiguel-Ramos, B. Díaz-Durán, J.M. Escolano, J. Olivares, M. Clement, T. Mirea, E. Iborra Sensors and Actuators B: Chemical 222 Elsevier 2016 688-692 10.1016/j.snb.2015.08.120*

6 *"Influence of liquid properties on the performance of S0-mode Lamb wave sensors II: Experimental validation" T. Mirea, V. Yantchev, J. Olivares, E. Iborra Sensors and Actuators B: Chemical in press Elsevier 2016 10.1016/j.snb.2016.01.131*

7 *"Tungsten oxide layers of high acoustic impedance for fully insulating acoustic reflectors" M. DeMiguel-Ramos, B. Díaz-Durán, J. Munir, M. Clement, T. Mirea, J. Olivares, E. Iborra IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control in press IEEE 2016 10.1109/TUFFC.2015.2498968*

8 *"Discrete microfluidics based on aluminum nitride surface acoustic wave devices" J. Zhou, H. F. Pang, L. Garcia-Gancedo, E. Iborra, M. Clement, M. De Miguel-Ramos, H. Jin, J. K. Luo, S. Smith, S. R. Dong, D. M. Wang, Y. Q. Fu Microfluid Nanofluid 18(4) Springer 2014 537-548 10.1007/s10404-014-1456-1*

9 *"Characterisation of aluminium nitride films and surface acoustic wave devices for microfluidic applications" J. Zhou, M. DeMiguel-Ramos, L. Garcia-Gancedo, E. Iborra, J. Olivares, H. Jin, J.K. Luo, A.S. Elhady, S.R. Dong, D.M. Wang, Y.Q. Fu Sensors and Actuators B: Chemical 202 Elsevier 2014 202 10.1016/j.snb.2014.05.066*

10 *"Growth of Carbon Nanotube Forests of Metallic Thin Films" J. Olivares, T. Mirea, B. Díaz-Durán, M. Clement, M. DeMiguel-Ramos, J. Sangrador, J. De Frutos, E. Iborra Carbon 90 Elsevier 2015 9-15 10.1016/j.carbon.2015.03.058*

11 *"Carbon nanotube growth on piezoelectric AlN films: influence of catalyst underlayers" T. Mirea, J. Olivares, M. Clement, M. DeMiguel-Ramos, J. de Frutos, J. Sangrador, E. Iborra RSC ADVANCES 5 Royal Society of Chemistry 2015 80682-80687 10.1039/c5ra16840f*

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Conferences

1 *Conference, Oral Presentation DAVOS DIAGNOSTICS AG Point of care specific IgE measurements in ten minutes 22/06/2013 World Allergy and Asthma Congress 2013,Milan Scientific community (higher education, Research) - Industry - Civil society - Policy makers Worldwide*

2 *Oral presentation to a scientific event DAVOS DIAGNOSTICS AG Rapid measurement of binding affinities with evanescence biosensor technology 13/03/2013 World Immune Regulation Meeting VII, Davos Switzerland Scientific community (higher education, Research) - Industry - Civil society - Policy makers 200 Worldwide*

3 *Oral presentation to a scientific event DAVOS DIAGNOSTICS AG Evanescent wave fluorescence technology: substituting ELISA with fast real time background free diagnostics 10/06/2013 Research Center Borstel visit in Davos Scientific community (higher education, Research) 40 several*

4 *Oral presentation to a scientific event DAVOS DIAGNOSTICS AG Rapid measurement of binding affinities with evanescence biosensor technology 13/03/2013 Davos, Switzerland Scientific community (higher education, Research) 800 international*

5 *Poster DAVOS DIAGNOSTICS AG Measurement of human IL-10 and allergen specific IgE using the evanescence biosensor technology 13/03/2013 World Immune Regulation Meeting VII, Davos Switzerland Scientific community (higher education, Research) - Industry - Policy makers 200 Worldwide*

6 *Poster DAVOS DIAGNOSTICS AG EVA-sensor: A novel biosensor for performing rapid diagnostic tests 13/03/2013 World Immune Regulation Meeting VII, Davos Switzerland Scientific community (higher education, Research) - Industry - Policy makers - Medias 200 Worldwide*

7 *Poster DAVOS DIAGNOSTICS AG Evanescence biosensor technology for fast and quantitative measurement of proteins and nucleic acids 13/03/2013 World Immune Regulation Meeting VII, Davos Switzerland Scientific community (higher education, Research) - Industry - Policy makers 200 Worldwide*

8 *Poster DAVOS DIAGNOSTICS AG Development of a novel evanescence biosensor for sensitive and quantitative measurement of proteins 29/08/2013 Satellite Meeting SM5 of the 15th International Congress of Immunology, Perugia, Italy Scientific community (higher education, Research) 50 Worldwide*

9 *Poster DAVOS DIAGNOSTICS AG Rapid quantification of specific IgE in serum using an Evanescence Biosensor 29/08/2013 Satellite Meeting SM5 of the 15th International Congress of Immunology, Perugia, Italy Scientific community (higher education, Research) 50 Worldwide*

10 *Poster DAVOS DIAGNOSTICS AG Rapid aptamer-based assay for the detection of pathogenic bacteria 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

11 *Poster DAVOS DIAGNOSTICS AG Comparative measurements of allergen specific antibodies using the evanescent field method 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

12 *Poster DAVOS DIAGNOSTICS AG Rapid quantification of antigen-specific serum immunoglobulins 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

13 *Poster DAVOS DIAGNOSTICS AG HPA-1a Allo-Antigen Typing in Whole Blood Discrimination of a Single Amino Acid Change in a Ten Minute One-Step Assay 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

14 *Poster DAVOS DIAGNOSTICS AG Detection of tryptophan and tryptophan metabolites by evanescence technology 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

15 *Poster DAVOS DIAGNOSTICS AG Blood, Sweat and Tears different biological fluids for use in evanescence biosensor tests 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

16 *Poster DAVOS DIAGNOSTICS AG Simple, Specific, Sensitive, and Rapid Detection of Toxoplasma Antibodies 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) - Industry 800 Worldwide*

17 *Poster DAVOS DIAGNOSTICS AG Replacing ELISA tests with ultra-rapid evanescence field-based technology 02/04/2014 World Immune Regulation Meeting VIII, Davos Switzerland Scientific community (higher education, Research) 800 Worldwide*

18 *Conference, Oral presentation UPM Rapid Aptamer based diagnostics for bacterial meningitis 15-20 September 2013 XX Conference on Liquid Crystals, Mikołajki, Poland Scientific community (higher education, Research) 80 International*

19 *Conference, Oral presentation UPM Liquid crystal biosensing of microorganisms 13-18 September 2015 16th Topical Meeting on the Optics of Liquid Crystals OLC'2015, Sopot, Poland Scientific community (higher education, Research) 80 International*

20 *Conference, Poster Presentation UPM Functionalized iridium oxide as detection surface in gravimetric biosensors 10-13 May 2015 4th Conference on Bio-sensing Technology 2015, Lisbon, Portugal Scientific community (higher education, Research) - Industry 150 International*

21 *Conference, Oral presentation UPM Aptasensor for detection of Neisseria meningitidis and Streptococcus pneumoniae cell surface-associated proteins Submitted Biosensors 2016, 26th Anniversary World Congress on Biosensors, 5-27 May 2016, Gothenburg, Sweden Scientific community (higher education, Research) - Industry 200 International*