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Multisense Chip

The lab-free CBRN detection device for the identification of biological pathogens on nucleic acid and immunological level as lab-on-a-chip system applying multisensor technologies

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Final Report – Publishable Summary

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Project coordinator:	**Dr. Claudia Gärtner**
Tel:	**+49 (0) 03641 34705 20**
Fax:	**+49 (0) 03641 34705 90**
E-mail:	**Claudia.Gaertner@microfluidic-ChipShop.de**
Project website address:	**www.Multisense-Chip.com**

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1 “Multisense Chip” project abstract

The goal of the “Multisense Chip” project was to build an integrated “sample-in-result-out” analytical system for the detection and identification of biological pathogens. Two user scenarios were covered within the project resulting in two different systems: A **“one-time measurement system”** enabling the analysis of samples on demand and a **“permanent monitoring system”** ensuring a permanent monitoring of potential contamination of sensitive areas with biological pathogens. The “Multisense Chip” systems with a lab-on-a-chip as core component allows for a **detection of biological pathogens on molecular and immunological level**.

2 “Multisense Chip” Excecutive Summary

The goal of the “Multisense Chip” project was to build an integrated “sample-in-result-out” analytical system for the detection and identification of biological pathogens. Two user scenarios were covered within the project resulting in two different systems: A **“one-time measurement system”** enabling the analysis of samples on demand and a **“permanent monitoring system”** ensuring a permanent monitoring of potential contamination of sensitive areas with biological pathogens. The “Multisense Chip” systems with a lab-on-a-chip as core component allows for a **detection of biological pathogens on molecular and immunological level**.

Main characteristics of the “Multisense Chip Analyzer” are a “sample-in-results-out” operation manner, an extremely easy handling even suited for untrained personnel, and finally a fast response time of roughly 60 minutes for the overall process, and finally a lab-free approach. The “Multisense Chip Analyzer” can be operated at the place of need, what differs from the current state of the art, were all biologically challenging steps require equipped laboratories and trained staff.

At first, a set of typical B-agents for e.g. terroristic attacks was chosen as target species that the “Multisense Chip Analyzer” needs to detect comprising *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, *Burkholderia pseidomallei*, Brucella melitensis, *Brucella abortis*, *Coxiella burnetti* and *Bacillus anthracis*.

To cope with the envisaged “sample-in-result-out” operation procedure, the **“Multisense Chip Analyzer”** includes the complete process chain from sample collection followed by sample transfer into the lab-on-a-chip system. The subsequent processes on chip encompass sample preparation for direct extraction of target molecules of various kinds of matrices, the biochemical detection method, and, finally, the readout via different sensor technologies.

Keys to reaching the above project goals were **advanced sensor technologies**, **lab-on-a-chip concepts** with its intrinsic advantages of miniaturization and **innovative instrumentation**. Several enabling technologies based on micro- and nanotechnologies came into place for the realization of the various components of the lab-on-a-chip system, biochemical processes, as well as scientific instrumentation. Information & communication technologies coping with the control of the system, the analysis and presentation of the analytical results, as well as with the communication of eventual findings to first responders and respective authorities were involved.

Due to the multifaceted tasks resulting from bio-analytical, microfluidic, engineer-technical, communication-technical, and, finally, end-user based requirements, the “Multisense Chip” project was composed by a high multidisciplinary project team. Taking the competencies of the “Multisense Chip” project partners in mind, it was ensured that their complementary expertise and overlapping know-how in the critical project paths not only covered the above demand of the project, but directly implied a contingency plan to guarantee a smooth course of the project.

3 Summary description of the project context and the main objectives

The terroristic attacks with non conventional weapons as the B-agents attacks in the US of October 2001, or the avian flu in Asia and Europe, or the Ebola virus outbreaks in West Africa demonstrate not only the limited capacities of the different national analytical labs, but also highlights the handling of such major threats and the proper response to them being massively hindered by the lack of rapid, safe and portable detection and identification methods to securely determine an infection or contamination of people, animals, food or sensitive infrastructure.

Most of the presently available commercial detection methods for biological pathogens are based on three fundamental methods and have different drawbacks. Microbiological and immunological procedures are usually quite cost-efficient, but can only be carried out within a suitably equipped laboratory infrastructure. Furthermore, they are time-consuming and not very sensitive. Analytical instruments based on nucleic acid detection are very specific with respect to the different biological pathogens and also very sensitive, but usually they cannot be used in a mobile environment, are time-consuming and complex, and can only be handled by trained staff. Sample preparation and analysis still need at least several hours, which is unacceptable in critical situations like those involving victims of B-agents and critical infrastructure.

The potential risk of airborne pathogens illustrates the urgent need for sensitivity: airborne pathogenic bacteria require sample enrichment from a large volume of air, its transfer into a small volume of liquid, followed by a detection method as sensitive as possible, ideally reaching a detection limit of one DNA copy per assay. Highly infectious bacteria like *Francisella tularensis, Brucella melitensis* or *Coxiella burnetii* have a low infectious dose. Therefore, any device assumed to be used in continuous environmental monitoring should be able to detect minute amounts of hazardous microorganisms in air samples. To ensure early warning, highly efficient air sampling and concentration of airborne particles are critical steps in continuous surveillance.

The final goal of the Multisenses Chip project was the development of analytical instruments to carry out with confidence the detection and identification of biological pathogens on the molecular and immunological levels.

The systems are based on a control instrument and a lab-on-a-chip as a consumable. They do not only carry out the detection reaction, namely the PCR and immunoassays, but also the sample enrichment and the extraction of the target molecules. Finally, the systems also carry out the detection via electrochemical and optical means. All functions after sample uptake, concentration and transfer are integrated on-chip to avoid all manual handling steps as well as the need for equipped laboratories and trained personnel. This means a “sample in, result out” system is at hand, directly highlighting the analytical results on the molecular and immunological levels.

The systems developed in the Multisense Chip project cover two user scenarios:

1. Permanent monitoring of airborne pathogens: In this user scenario bacterial pathogens are spread into air, meaning that large volumes of air have to be sampled, target

molecules have to be transferred in a liquid phase that is concentrated and a defined volume is moved on the lab-on-a-chip device.

2. **One-time measurement of liquid and swab samples:** This scenario implies that bacteria or other pathogens are exposed on surfaces like letters inside envelopes, dispersed onto food or in liquids/beverages and that the suspicious sample will be collected with a swab or directly as liquid. This required the establishment of a methodology to collect the sample in a standard manner, to extract the sample from the swab, and to quantitatively transfer the sample from the sampling vessel to the chip.

The analytical pathway of both instruments and technologies applied in the final integrated instruments are summarized in Figure 1.

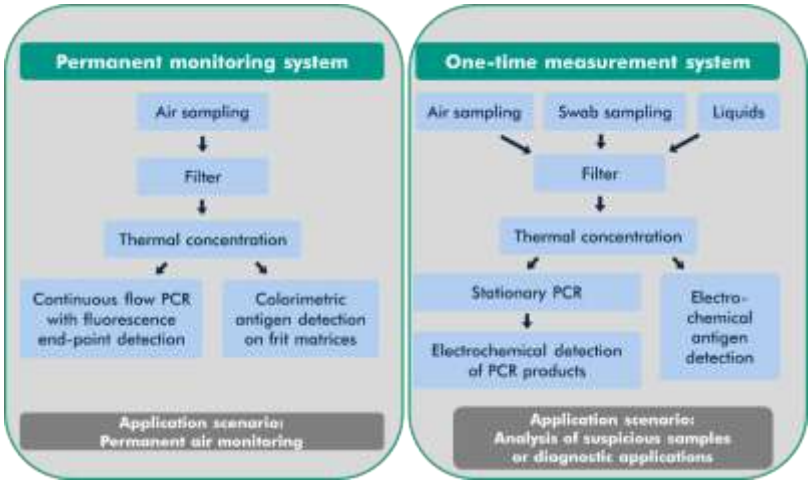


Figure 1: Analytical pathway and methods applied for both user scenarios of the Multisense Chip project

To cover both user scenarios “permanent monitoring” and “one-time-use” different lab-on-a-chip solutions and two control instruments to run the chip were developed.

The overall project target, the realization of a “sample in, result out” system for the detection and identification of biological pathogens on the molecular and immunological levels was achieved by a combination of the following enabling technologies:

1. **Nano- and microfabrication technologies:** Nano- and microfabrication technologies enabled a miniaturization of the system, a massively reduced sample and reagent consumption, a decrease in the overall reaction time, the integration of several biological steps, a parallel processing of two detection reactions without any manual handling steps after loading of the sample.
2. **Innovative detection technologies:** Electrochemical sensors, based on fabrication strategies developed in the course of the project were directly integrated on the plastic chip and used for the detection on the immunological and molecular levels in an extremely sensitive manner.
3. **Biological process steps:** A prerequisite for the detection bacterial DNA, which must also be very specific to avoid false positive reactions, is the adequate construction of highly specific DNA primers and probes. Suitable reagents were constructed and validated for the following six bacterial agents of category A&B: Francisella tularensis,

Yersinia pestis, Brucella melitensis, Burkholderia mallei, Burkholderia pseudomallei and Coxiella burnetii.

4. **Scientific instrumentation:** Advanced technologies and novel solutions were combined to both final instruments that cover the complete analytical process: control the fluidic, thermal, electronic and optical functions of the lab-on-a-chip device and, as an add-on, covers sample enrichment from air.

The overall system was realized via the combination of all mentioned technologies, merging engineering science with the biological and diagnostic fields, advanced sensor technologies, and scientific instrumentation. This combination of different disciplines to a multidisciplinary network of enabling technologies posed the highest technological challenge.

The overall systems comprise the following functionalities, which are implemented in the following manner:

Air sampling: For the sample uptake from air, the Coriolis system from Bertin was used as a basic ingredient and adopted to the special needs of the project namely a high recovery rate enabled by long sampling and special process conditions: A dry air sampling process and a permanent operation mode for the system were developed and successfully implemented achieving a significantly higher recovery rate.

Swab sampling: For the sample uptake of suspicious components swabs were the method of choice. A special sampling vessel was developed that is prefilled with buffer in which the swab is inserted after sampling. The innovative features are two characteristics: The sampling vessel can directly mounted on the lab-on-a-chip device with the help of an a proper integrated fluidic interface and due to an intrinsic septum, the vessel can be removed without any contamination risk allowing to send a fraction of a sample to other analytical sides. The swab was transferred in a reaction vessel prefilled with sampling buffer.

Liquid samples: Liquid samples were directly gathered in a sampling vessel and mounted to the instrument. After finishing the analysis of the sample, the vessel can be disposed or be sent to other analytical sides.

Sample preparation and concentration: To isolate DNA and antigens and to reduce the overall sampling volume a special sample preparation module was developed that combines concentration and sample preparation. For sampling a certain volume was transferred to the concentration module and the overall volume of the sample was reduced to a defined volume. During this step not only the DNA and antigens for the molecular and immunological assay were isolated but also the sample was concentrated.

Immunological detection: On chip, a standard immune reaction took place. The specific antibodies were presented in two manners: In the **Permanent Monitoring System** (PMT) they were immobilized on filter frits that allow for a sample concentration since the complete sample is flushed through these frits as porous elements catching the respective target molecules. In the **One-time Measurement System (OTM)** the antigens were attached to the gold surface of the electrochemical detection electrodes and the sample was flushed over the array.

Detection on molecular level: After the sample preparation for the DNA pathogens, a PCR (Polymerase Chain Reaction) for the amplification of the target DNA was carried out using a

continuous flow approach in the **Permanent Monitoring System** and a stationary approach in the **One-time Measurement System**.

Electrochemical detection: This detection technology was used in the **One-time Measurement System** and required an additional detection reaction in order to generate an electrochemical signal. The electrochemical set-up was read out by electrochemical sensors. For this assay for both detection levels, the molecular and immunological detection, an enzyme label and substrate were employed and the generated product was detected using an novel integrated pluri-potentiostat coping with 64 parallel detections.

Fluorescence detection: Fluorescence detection was used in the **Permanent Monitoring System**. This detection method required a fluorescence label that was integrated in the DNA during the PCR.

Photometer detection: The last detection method was used to detect the antigens in the **Permanent Monitoring System**. The basic function is equal to the electrochemical detection. Detection antibody were immobilized on a filtration frit after binding the antigen a detection antibody followed by a detection solution were exposed to the frits. In case of positive detection the color change (reduction of light transmission) of the frit was measured using a photometer.

IT – Software & communication: Efforts on the IT side were made on three levels: First of all, the control software for the instruments to run the chip was successfully established on both instruments. The second level was the software for the read-out of the detection reaction. The third level was the information and communication software that was developed and implemented to the system.

System integration: All functionalities of the instrument ranging from air collection, fluid and thermal management, optical and electronic read-out, data analysis and the communication tool to e.g. first responders and others authorities were successfully integrated into both systems, the **Permanent Monitoring System** and the **One-time Measurement System**.

Finally two integrated systems were at hand to cope with the Multisense Chip project goal, namely to analyze biological samples in a sample-to-answer fashion without any manual interaction besides the sample introduction. This demonstrates that besides the technical challenge of the realization of the consumable lab-on-a-chip device and the respective instrument all biological tasks were combined on the platform and that the multidisciplinary team achieved a working system that was successfully evaluated with first responders.

4 Main S&T results/foregrounds

The “Multisense Chip Analyzer” is an easy to handle but technologically complex system combining a multidisciplinary approach from engineering, science and information and communication technology in order to cope with a blead-to-read fashion of the analysis of biological samples. This required development on technical level in order to realize the lab-on-a-chip system and the respective instrument, on biological level to establish all steps from sample uptake over sample preparation to the final assay, and on the communication level to allow for spreading the analytical results in a predefined network of first responders according to a fixed communication chain. Finally, ethical aspects were covered in detail keeping the later user scenario touching the diagnostic area in mind: Critical items such as the potential use of animal or human samples were discussed and respective documents were put in place. The evaluation of the system revealed, however, that neither human nor animal material was used. Furthermore, the handling of restricted information and hazardous substances was covered within the ethical work package.

In order to cope with this multidisciplinary challenge a consequent risk minimization strategy was implemented. Core element of this risk minimization were two elements: A **modular approach** and **standardization**: Basis of the **modular approach** was the division of the overall task in technical module and sub-modules allowing for a parallel development e.g. of single biological steps, evaluating instrument and fluidic behavior separately from the biology etc. In addition, parts of the instrument as well as the different detection technologies were realized and evaluated in parallel. This ensured a concerted development of all biological process steps on technical and biological level. Apart from the modular approach, a **standardization concept** was implemented defining outer geometries of the lab-on-a-chip device and principle functional elements of the required consumables and interfaces to the instrument. This enabled a parallel development of instrument modules and the lab-on-a-chip device.

The line of action following the requirement specification and the time and risk saving actions are summarized in the action tree in the Figure 2.

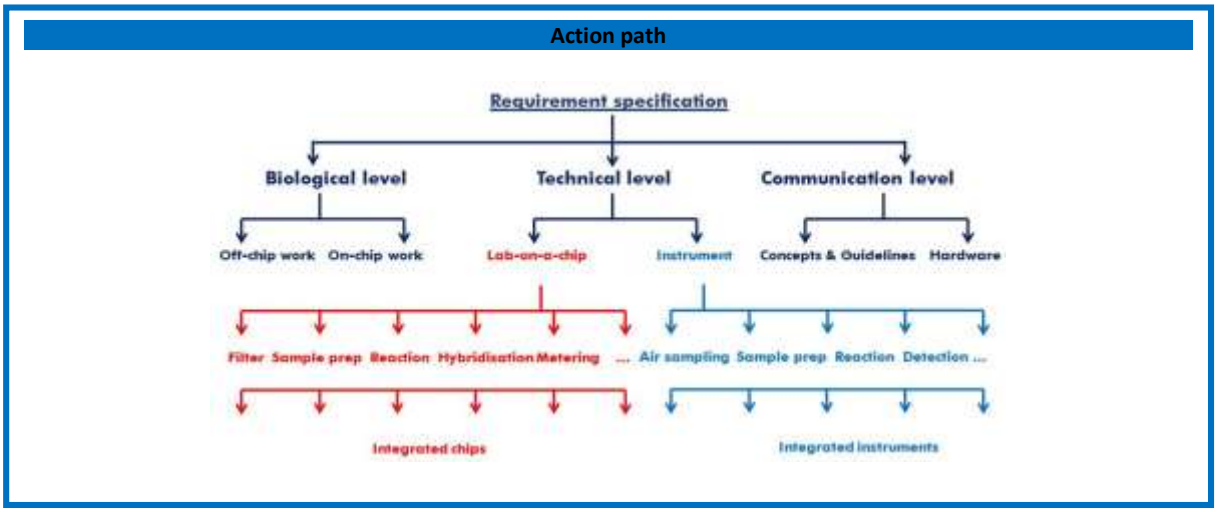


Figure 2: Actions path following the requirement specification

Enormous effort on the S&T level in the different areas ended up not only in two integrated systems but also in a wide variety of innovations that are on their way to commercial use. This covers the fields of sensor technology, biological assay development, microfluidic devices and systems as well as respective instruments, the communication technology and finally system integration and system validation. The interdependency of the different tasks was a organizational challenge in order to ensure the parallel efficient development and to draw conclusions from e.g. biological results for the microfluidic device choice.

The **first and second project year** were dedicated towards the different sensor technologies, chip and instrument development, the implementation of all relevant biological reactions on chip, namely sample preparation, nucleic acid extraction, immunoassay, and the molecular assay on lab-on-a-chip modules and to perform the assays for all targets organisms defined within the requirement specification. The communication strategy with first demonstrations was further advanced.

The **third project year** was characterized by several success stories. Highly sophisticated bio-analytical steps were successfully translated into a “microfluidic language” by streamlining these techniques, by minimizing bulky sample volumes without loss of analytes, and by drastic reduction of test time taking advantage of the short diffusion pathways within microfluidic systems.

During the **last project period**, the work on the final instrumentation and chips as well as the assay optimization continued. The biological protocols for DNA isolation and concentration, immuno- detection, and on-chip PCR were further optimized on chip. The finalization of the detection protocols for amperometric and electrochemiluminescent DNA and antigen detection led to the development of the final sensors for DNA and antigen- detection in the One-time Measurement System. Gold and carbon electrodes were manufactured using the “contact through” screen printing technology that was developed in the course of the project and the electrode surface was functionalized successfully with the DNA and antigen detection probes. The chip designs for permanent monitoring and one time measurement were finalized; the chips were produced and all detection technologies were integrated. The instruments dealing with the permanent monitoring and the one-time measurement scenarios were developed and finalized. This included not only the development of the hardware but also the control software consisting of the control modules for fluidic control, detection, and communication and the validation of the instrument parameters. Using the chips and the corresponding instruments – the Permanent Monitoring System (PMS) and the One-time Measurement System (OTM) – fluidic protocols were developed and validated and the biological protocols were transferred from module level to the integrated chips. Next the chips and instruments were tested with biological samples and finally validated on biological level. As a final step the instruments were tested by potential end users, giving important advices for system improvement.

4.1 Main S&T results/foregrounds in detail

4.1.1 S&T area ASSAY DEVELOPMENT WP 2

Assay definition and assay development were the starting points, since with the definition of the assays themselves, the targets, the antibodies and first process chain of the biological steps was set. A biological flow chart was developed that was transferred in a fluidic flow chart defining sequences, volumes, timing and thermal or electrical actions.

The biological processes were all developed on chip and for benchmarking the gold standard technology was applied.

Sample uptake as first step of the analytical was developed. For **solid samples** swabbing was combined with the use of a specially developed sampling unit that can be prefilled with reagents, allowing for a sample release and the direct hook on the lab-on-a-chip device. Respective release buffer systems allowed for a nice solubilization of samples deriving from various surfaces. Air sampling was implemented with a technical approach from Bertin using a sampling unit based on Coriolis forces. Since water was found suited for the release of the pathogens from the surface of the sampling unit no further actions on this sampling tasks from the biological point of view was necessary. Liquid sampling was achieved with the same sampling vessel as used for solid samples but not pre-filled, alternatively the liquid was directly introduced on chip. No further actions were necessary from the biological development aspect.

Sample concentration and **sample preparation** were main biological tasks since large volumes deriving from air sampling came into place and also liquid samples taking environmental screening and contaminated beverages into account were of interest. For both, air sampling and large liquid volumes, a novel procedure was applied, that combined an engineering innovation with a biological process development: Large liquid sample volumes were subjected to a vacuum and heat assisted process resulting in a volume minimization by an order of magnitude in a couple of minutes without harming the biological components – in particular the immunoassay targets: The concentration from 3 ml to a few 300 µl in a six minutes was an achievement matching perfectly the task to be achieved. A second biological process for the same task focusing the molecular target was developed that started with lysed material where the nucleic acids were free-floating: This material was moved over a band of specially prepared magnetic beads handled as a column in one on the Multisense Chip modules consisting of chip and instrument. Finally, this procedure showed an impressive concentration effect and a competent nucleic acid extract for real time PCR. For comparison the gold standard Qiagen column was used, and superior results could be achieved with the Multisense Chip method developed.

PCR on chip was an interesting feature, since this massively influence the overall timing as well as the size and potential complexity of the final lab-on-a-chip device. Firstly, as starting point all PCR reactions were developed on real time PCR cyclers being used also as benchmark systems. Three different PCR principles on chip needed to be benchmarked an all processes had to be implemented on these systems. One special challenge was that one cycling protocol in respect of number of cycles, temperature, cycling times needed to fit all 8

different microorganisms and still needed to be sensitive and specific. Figure 3 shows that this goal was nicely reached on chip, despite development work is still ongoing.

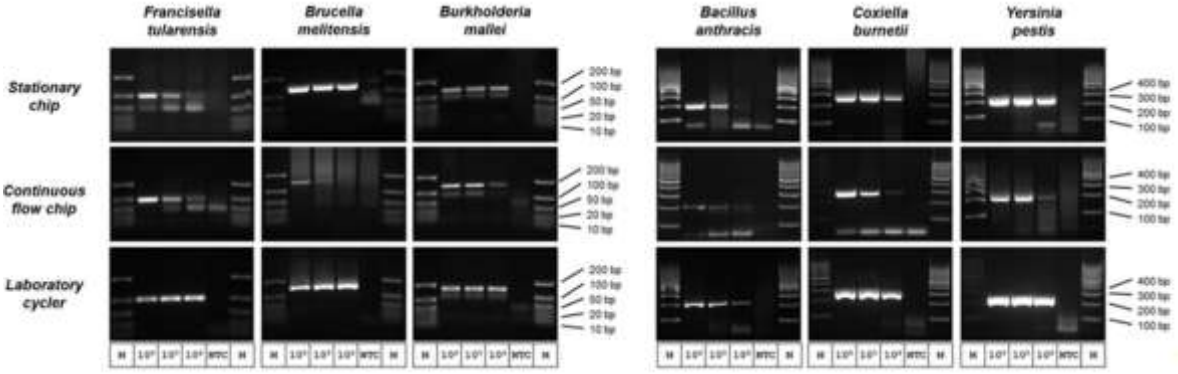


Figure 3: “One-protocol-fits-all” proof-of-concept for on-chip-PCR

The first PCR approach being a standard PCR just transferred on chip, called “stationary PCR”, makes use of the normal heating and cooling procedure of the instrument was applied. Due to the minimization of instrument, heating zone and interfaces and making use of fast cyclers a time gain was achieved. The **“stationary PCR”** approach was combined with real time fluorescence-based detection as well as in the integrated system with electrochemical end-point detection. For the real-time approach common PCR protocols fitting all different targets reached similar results as target specific PCRs in a real time thermocycler. A roughly 30 min protocol compared to 1 – 1.5 hours in a conventional system demonstrated the potential of the technology. This gives potential for further application besides the integration in the Multisense Chip integrated system. Also the electrochemical detection of all different PCR targets in an end-point manner was achieved.

An **“oscillating”** or **“Boyle Mariotte-PCR”** was the second technological approach towards implementing the PCR on chip. Equipped with a real time detection this approach gave 10 – 15 minutes cycling times and the cycles could be freely programmed by pumping liquid back-end-force over heating zones fixed in the instrument.

The **“continuous flow PCR”** moves the sample over fixed heating zones of the instrument in a long meandering channel that defines the number of cycles. The different targets are stacked after each other and move over the heating zones of the instrument sequentially. The read-out is achieved by end-point fluorescence detection. Single PCRs could be implemented on chip to have an overall cycling time for a 40 cycle PCR of 10 minutes, taking the one-fits-all protocol into account much longer PCR timing was necessary. Since an internal control was used as second PCR but no multiplexing was done, the PCR reactions for the different targets were stacked, meaning on PCR follows the other locally in the meandering PCR channel as well as time wise at the final optical detection area. In order to cope with the large surface area and the potential of unspecific binding special PCR recipes needed to be developed.

The chieved timeline for a 40 cycle PCR for stand alone on-chip PCR-modules is shown in Figure 4 as well as detection limit with the respective chip modules used.

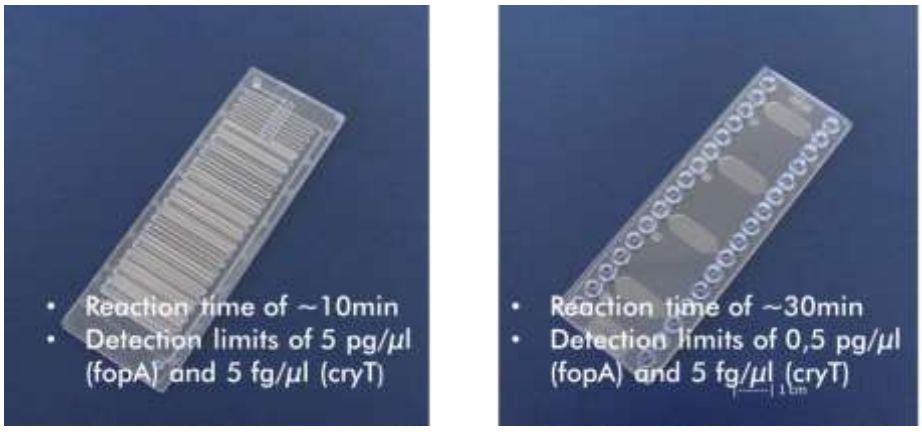


Figure 4: Chip modules and achieved time for on-chip continuous flow (left) and stationary PCR (right)

Technology choice PCR: The footprint being necessary for the "stationary PCR" on chip is relatively small, reagent integration in the cavities themselves is easy and therefore parallelization can be nicely achieved. This was the reason for the technology choice for the one-time measurement systems were all different reagents needed to be integrated on chip. The easy permanent operation of the continuous flow PCR approach and that due to permanent sampling the overall PCR time was not so critical the continuous flow PCR was chosen for the permanent monitoring system.

Immunoassay – frit based approach: The special challenge of the immunoassay for the permanent monitoring system was that one protocol needed to fit all assays but in particular that also if a negative sample is flushed over the surface, this biological active surface still needs to be able to detect the target molecules once it is flushed. To cope with this challenge a frit base approach was used. The respective chip module is shown in Figure 5.

These frits are porous elements placed in the liquid flow of the sample which is moved in a 3D fashion through this matrix. On the frit itself the antibodies are linked in a stable manner. Both, the antibody presenting frits and the assay process itself resulting in a colorimetric read out were successfully implemented. Through a special development of the assay reagents, the immobilization technology and the fluidic protocol it could be successfully demonstrated, that after 8 days of flushing negative samples a positive detection occurs as envisaged once the target molecule is flooded as shown in Figure 6.

Figure 5: Chip used for frit-assay development

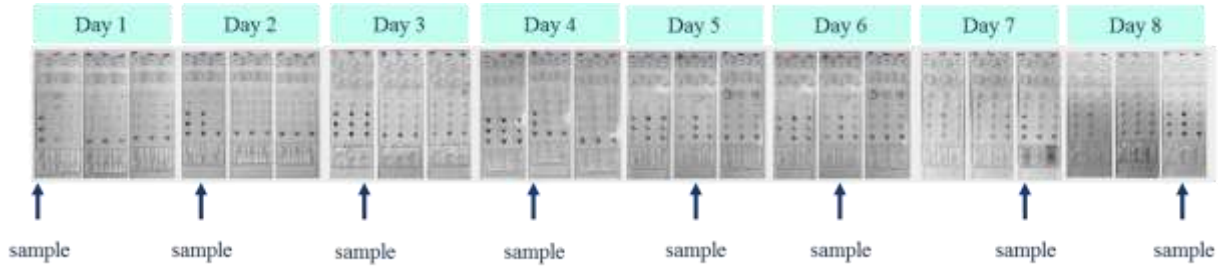


Figure 6: Results with the immunoassay on frits after flushing of negative sample over a longer period indicating a positive results once target analyte is flushed

Hybridsation assay – electrochemical assay for molecular and immunological targets: For the hybridization assay a wide variety of different process steps needed to be implemented: The immobilization process for the target molecules on the electrode array, the special "background-molecules" attached to the target, and further molecules binding on the electrodes to avoid unspecific binding covered in the sensor task, and the implementation of the biological process itself were the major efforts. The choice of the best suited reagents for the assay was important taking envisaged shelf life a one year into account. Two main achievements besides the complete implementation of the process can be highlighted: Firstly an achieved detection limit of 31 bacteria / ml for *Francisella tularensis'* LPS antigen and the minimization of the hybridization time from formerly hours, 20 min in a first improvement step to finally 2 min.

Taking the envisaged use into consideration **reagent storage** is a major issue. Two different approaches were developed. Firstly, the dry storage of the complete master mix requiring special procedure and additives for the polymerase to be able to get a long-term stable enzyme after the lyophilization process is one challenge that was successfully addressed. Secondly, the reagents for the hybridization assay and the frit based immunological assay. The reagents need to be stored on chip in a ready-made fashion without requiring additional dilution steps. Drawback is the shelf life of the diluted enzyme for the detection reaction. Stabilizing agents were developed and implemented on chip to show a several months life time compared to immediate reactivity loss in a couple of hours from standard reagents.

In brief, the biological tasks were completely implemented on chip covering sample enrichment, clean-up as well as the analytical detections on immunological and molecular level in a sensitive and quick manner. The topic of reagent storage in dry and liquid format was implemented. Single results of this work will be exploited as well as the final implemented assay that will take further development work.

Summary of the S&T area ASSAY DEVELOPMENT WP 2 main outcome and exploitable results:

- **Biological and fluidic flow chart for the one-time and permanent measurement scenario**
- **Assay definition**
- **Reagent choice**
- **Reagent storage**
    - **Dry storage:** Lyophilization process and long-time storage establishment for the complete master mix

- **Liquid storage:** Integration of all assay reagents in blisters on chip for liquid storage and developing of stabilizing reagents for the immunoassay components.

- **Achievement on various aspects of PCR**
  - Biological processes for three different technical PCR processes were successfully implemented on chip: Stationary PCR, continuous flow PCR and Boyle-Mariotte (oscillating PCR)
  - Fast PCR processes ranging from 10 – 30 minutes depending on technological choice achieved
  - One protocol fits all implemented: Common PCR cycling procedure for all targets successfully implemented
- **Achievements on immunological assay**
  - A frit-based approach combining sample enrichment and colorimetric detection was successfully implemented
  - One protocol fits all implemented: A common immunoassay protocol for all reactions embedded on chip was achieved
  - Permanent use of biological reactive surface: The assay process and the special immobilization strategy of the antibodies on the frit surface resulted in a durable surface that can be flooded permanently with negative samples and finally can detect once the target molecules arrive.
- **Sample preparation**
  - Sample concentration achieved with magnetic beads next to the clean-up effect
  - Sample enrichment with a combination of vacuum and temperature without harming the biological targets established.
- **Process integration:** Implementation of the complete analytical process on the lab-on-a-chip device for the one-time and permanent-measuring system

4.1.2 S&T area SENSOR TECHNOLOGY WP 3

The development of a sensitive sensing technology based on electrochemical or electrochemiluminescence detection to be embedded on chip was the goal. Different work aspects needed to be covered: First task was the electrode development for both sensing technologies covering various design cycles (see Figure 7) and their final implementation in the integrated devices (see Figure 8). Secondly, new chemistries were set-up addressing the linker of the catcher molecules to be immobilized on the electrode surface, the material to cover the remaining electrode surface called “back-filler” to avoid unspecific binding, methods to amplify a signal either by multiplying through release of encapsulated particles and working using the enhancing effect of nanoparticles. The implementation of the process of screen-printed as well as lithographically made electrodes and their benchmark was a major task with the achieved aim to work with screen-printed electrodes that can be fabricated in a cost-efficient manner.

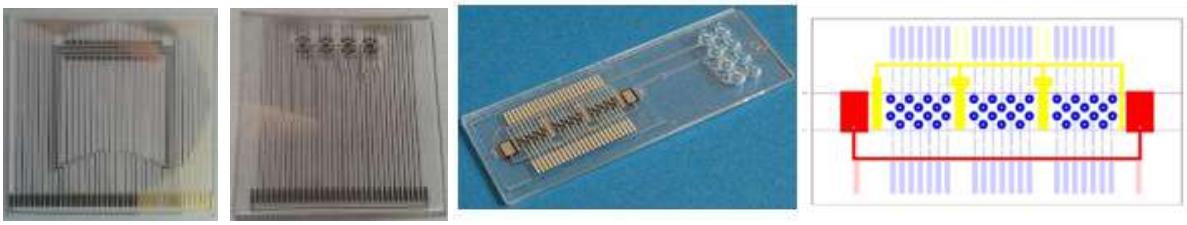


Figure 7: Evolution of the electrode design with rectangular and circular electrode arrangement (first and second from left), final electrode array for the tester set-up (third picture) and final electrode arrangement as scheme (right).

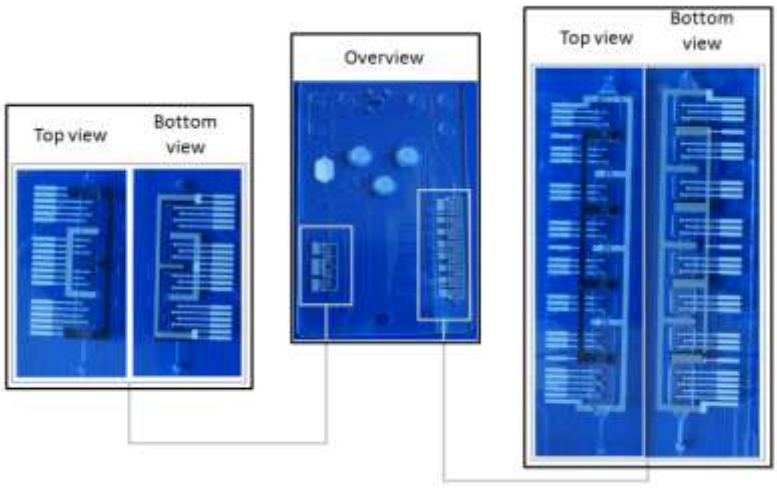


Figure 8: Electrode integrated in the fluidic platform, immunologic side (left) and DNA side (right)

Great progress in surface modification of the sensors for immunoassay detection of bacteria was achieved in the course of the project. The best results were obtained using lyotropic líquid crystals (LLC) hexagonal phase as a template for the immobilisation of the capture monoclonal antibody via thiolated alkyl molecules (DT2) containing a carboxy group. This surface chemistry resulted in decrease of non-specific adsorption, achievement of wide linear range (0-75 ng/mL) and low limits of detection (3.4 ng/mL) of antigenic LPS. Significantly, the approach was successfully applied for the detection of whole bacterial cell of *Francisella tularensis* as a model and a limit of detection (LOD) of 31 bacteria/mL was achieved.

An alternative surface chemistry based on diazonium salt for immobilisation of antibodies or DNA on gold or carbon electrodes was developed. LOD of 0.3 nM was obtained for *Francisella tularensis* DNA target using ECL detection on screen printed carbon electrodes. However, reproducibility of the measurement was low (R= 0.985). The reproducibility was improved by using $Ru(bpy)_3^{2+}$-liposome as ECL label (R = 0.994 and LOD= 0.4 nM).

Multiplex detection of eight different bacterial targets was demonstrated using DNA sandwich assay using horseradish peroxidase (HRP) as an electrochemical label. The results showed

that the designed probes for the complementary targets were very specific to their complementary probes and showed no cross reactivity. This approach was successfully tested for detection of PCR products of eight bacterial targets (Figure 9).

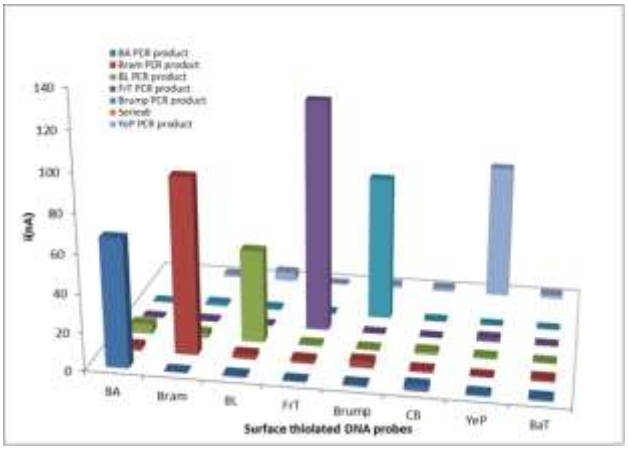


Figure 9: Amperometric measurement of eight PCR amplified pathogen targets and corresponding cross-reactivity. Single, thiolated probe and DT1 (Dithiol 16-(3,5-bis((6-mercaptohexyl)oxy)phenyl)-3,6,9,12,15-pentaoxahexa-decane) were co-immobilised in 1:100 ratio. Then, one PCR target at the time was introduced. The amperometric signal was measured using an HRP-labelled reporter strand; Bacillus anthracis (BA), Brucella abortis / Brucella melitensis (BrM), Bacteriophage lambda (BaL), Francisella tularensis (FrT), Burkholderia mallei / Burkholderia pseudomallei (BuM), Coxiella burnetii (CoB), Yersinia pestis (YeP), Bacillus thuringiensis (BaT)

In a subsequent step, all reagents were automatically delivered to the electrode array according to a script-based instruction program, using the automated microfluidic tester setup. The assay parameters were optimized and the required incubation time within the fluidic setup was reduced from originally 20 to 2 minutes for the target, which was most likely due to short diffusion ways within the microfluidic set-up. A 15-minute incubation was required for the labelled secondary antibody and, together with the automated washing steps after each incubation, the automated substrate addition, and the ultimate measurement, the overall assay lasted 22 minutes only.

Furthermore, the specificity of the immunosensor was clearly determined by investigation of the unspecific binding of the immobilized antibodies to *Yersinia pestis* derived antigens; no significant signal was observed for *Yersinia enterocolitica* subsp. *enterocolitica* and *Yersinia pseudotuberculosis* including its nearest subspecie *novacida*. Using 100µl sample, a very low LOD of 0.45 ng LPS was achieved. Moreover, the assay in the tester setup comprised of automated fluidic washing steps allowed for further reduction of assay time. Figure 10 shows a typical amperometric detection reaction for LPS.

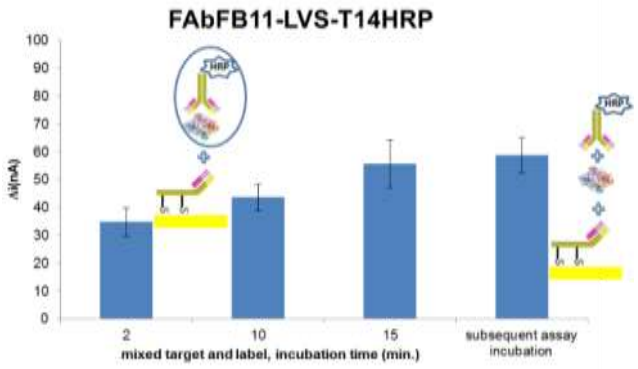


Figure 10: Step and Sweep (SAS) amperometric responses of the immobilised Fab fragment FB11 to fixed concentration (150 Bacteria/ml) of F. tularensis (LVS) at different incubation time of pre-mixed LVS target and secondary labelled antibody T14HRP and subsequent assay incubation. Each data point represents the average of nine measurements on three separate microchip array.

Electrochemiluminescence immunoassay of LPS was also covered. The LOD could significantly be improved by using Ru(bpy)32+ -encapsulated silica nanoparticles, because values down to 0.01 ng could be achieved.

A quantitative electrochemical measurement of 8 PCR products from pathogens on a single chip was performed. Low cross-reactivity and high selectivity was obtained in all cases.

Finally, screen printed gold electrodes were evaluated for DNA analysis and immunosensing more intensively. A higher signal could be observed on screen printed electrodes. In order to minimize error, normalization with respect to the active electrode surface needed to be done and the assay conditions were optimized.

Using electrochemical detection, six synthetic targets were measured on carbon arrays with LODs ranging from 0.6 to 1.2 nM. The signal could be considerably enhanced using dye encapsulated silica particles as labels.

Antibody fragments were generated against *Francisella* bacterial cells and dye encapsulated silica particles used as labels, achieving a detection limit of 45 bacterial cells/ml.

In brief: With the help of electrochemical- and electrochemiluminescence-tester set-ups both technologies were implemented successfully, both on screen-printed and lithographically made surfaces. Finally, a great sensitivity was achieved combined with even better results for the screen printed electrodes based on cheap manufacturing technologies and in the biological assay no cross-talk in the arrays with all targets embedded demonstrating the maturity of the technological result chosen.

Summary of the S&T area SENSOR TECHNOLOGY WP 3 main outcome and exploitable results:

- Know-how on electrochemical and electrochemiluminescence assays and chemical ways through reagent development to massively increase the sensitivity
- Screen printed sensors through analytical process development achieved to give superior results to costly lithographically made sensors
- Hybridisation process due to microfluidic approach minimized by orders of magnitude to 2 minutes

4.1.3 S&T area MICRO- AND NANOFABRICATION TECHNOLOGIES WP 4

According to the fabrication chain for the realization of polymer based lab-on-a-chip systems, several fabrication steps needed to be addressed in detail, mainly covering assembly technologies, surface functionalization and electrode fabrication.

construction of the sensor electrode arrays and there has been a steady improvement in screen printed technology that finally delivered electrode arrays with excellent reproducibility properties, with below 10% relative deviation.

Out of the different technologies initially proposed (screen printing and photolithography), screen printing showed to be as sensitive, reliable, and reproducible as photolithography, but with a fraction of the cost.

Of the different electrode array configurations initially proposed, the circular arrangement coupled with the circular PMT showed the best sensitivity and was therefore adopted.

Another couple of alternatives in sensor array construction, contact through and contact from top have been evaluated. Contact from top was the alternative with the lowest technical risk; however, it cannot deliver the electrode density required for this project. During the early phase of WP4, “contact through” technology was improved.

The most significant and promising technology developed subsequently allowed printing of electrode surfaces over the contact through vias. To achieve this, technical improvements in the drilling of the holes and filling them with electrical conducting paste were required. This configuration, called “plug electrodes” simplified the design especially on the fluidic side of the chip, thanks to the fact that all electrical contacts and tracks are directly on the opposite side of the chip.

Work on nanoimprinting technologies was somehow disappointing. The elevated cost and technical complexity hindered progress, and since the technological risk was still too large, it was decided to cancel this work sub-package at the end of year 2.

With respect to subtask 4.3 “Evaluation of heterogeneous integration and assembly technologies for microfluidic components”, the electrode arrays were integrated with the fluidics to form a single item. The performance of these integrated chips was successfully evaluated.

Mass manufacturing of electrode arrays and their integration into fluidic chips was attained during the last phase of the project and these sensors were be used to demonstrate the technology and can be considered as alternatives for the production of non-continuous CNBR detectors. The next figure (Figure 11) shows the screen-printed sensors working with two different materials, namely screen printed gold electrodes (SPGE) and screen printed carbon electrodes (SPCE) integrated in the Multisense Chip test chip. Different channel heights (150 µm and 250 µm) were evaluated together with the overall process resulting in a 250 µm deep channel to ensure a proper wetting of the electrodes.

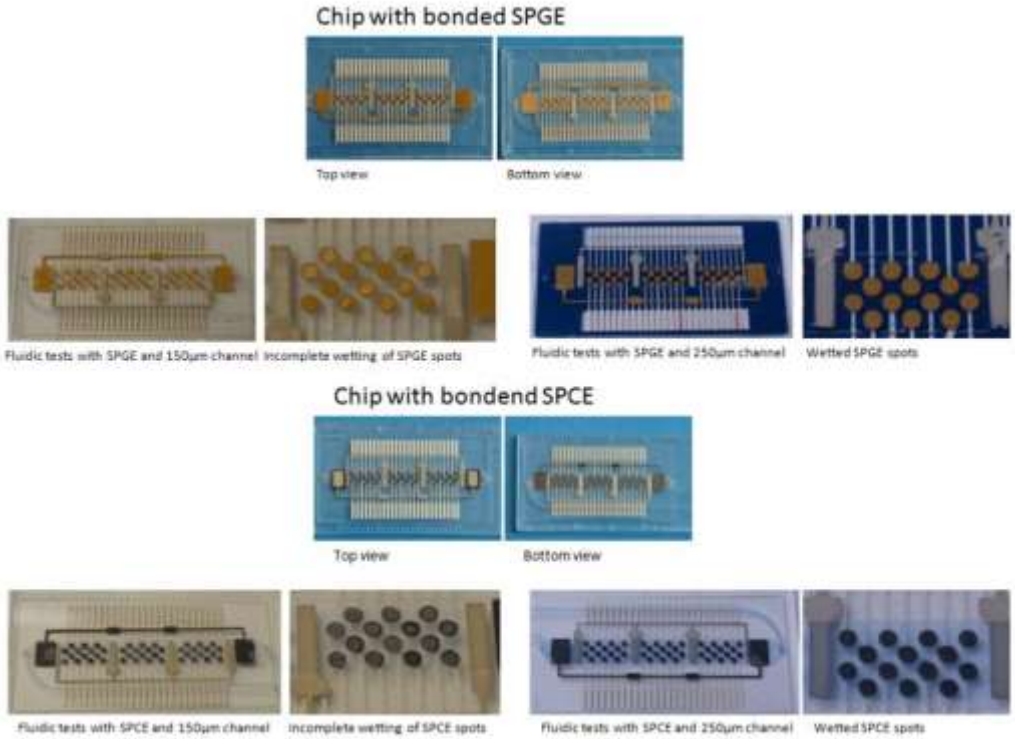


Figure 11: Assembly tests for sensor integration – screen printed sensors with gold (SPGE) and carbon (SPCE) electrodes on chips with 150 µm and 250 µm channel depth

In brief: Major achievements are the direct integration of the electrodes as hybrid elements working on an adhesive-free assembly principle and an adhesive-based assembling technology of hybrid elements made from different materials in polymer-based devices making use of a fluidic network. Immobilization technologies and respective chemical procedures allowed for a well-defined fabrication of the protein- and DNA-arrays by spotting. The most important outcome was the novel approach for the screen-printed electrodes: Moving from one-sided electrode array to double sided electrode structuring was achieved by a novel technology for implementing contracts through the electrode back-bone material. This technology can be implemented in a cost-efficient manufacturing manner and allows for a massive shrinkage of the electrode footprint, decreasing spotting cost by the order of the size reduction of the electrode device and chip cost through a smaller chip device.

Summary of the S&T area MICRO- AND NANOFABRICATION TECHNOLOGIES WP 4 main outcome and exploitable results:

- Novel fabrication technology for screen printed sensors to achieve contact through electrode arrays
- New integration concept of direct integration of screen printed sensors without the use of adhesives

4.1.4 S&T area MICROFLUIDICS AND MICROSYSTEM INTEGRATION WP 5:

Development of microfluidic modules and accessories was key for the later integration of all task on one lab-on-a-chip platform. Therefore, results for a straight forward exploitation are

lab-on-a-chip modules for the three implemented PCR technologies, sample preparation units, sampling vessels and fluidic interfaces.

The special PCR technologies “Continuous flow PCR” and “Boyle Mariotte PCR” resulting in extremely fast PCR due to avoiding the heating of the instrument but moving the sample over fixed heating zone achieving and instant temperature shift and therefore a direct reaction of the enzyme polymerase are shown in Figure 12.

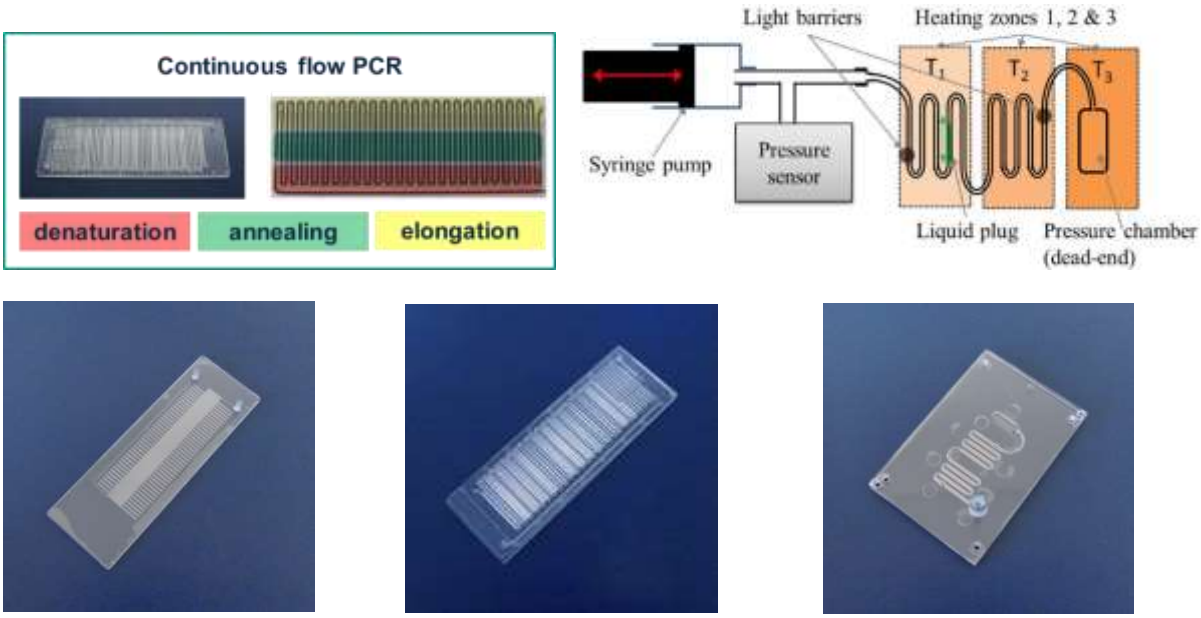


Figure 12: Functional principle of the continuous flow PCR (top left) and chip modules used (bottom left and middle) and functional principle of the Boyle Mariotte PCR (right left) and chip module used (bottom right)

Making use of microfluidics and in particular exploiting single modules requires several accessories like sampling vessels that can be directly hooked on the chips, fluidic interfaces, elements of liquid storage etc. All this modules were developed and realized within Multisense Chip and allowed for the fast biological process development and provide later on a quick exploitation prior to the integrated systems.

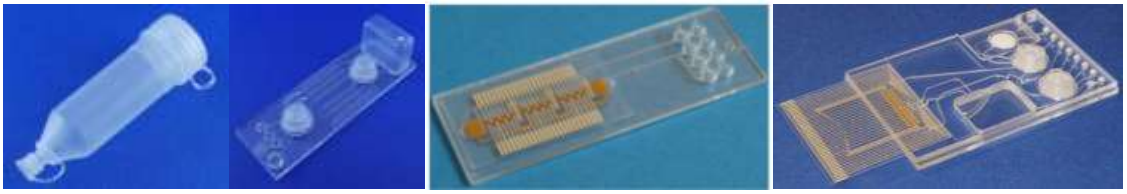


Figure 13: Sampling vessel (left), immunoassay module (second from left), hybridization module for electrochemical assay set-up (third from left) and immunoassay chip for electrochemical detection (right)

The main outcome in WP5 is the finalization of the chip designs Multisense design 14 and 15. The iterative progress from the prior Multisense design 12 (MUSE 12) to Multisense design 14 (MUSE 14) (Figure 14) gave rise to a novel mixing concept in terms of an additional frit. The advance from MUSE 13 to MUSE 15 (Figure 15) resulted in new blister positions, more blisters, and avoidance of storage of dry ingredients except the PCR mixture. In addition, the waste reservoir was larger and the sensors for the detection of DNA and antigens were integrated.

As a last point, it is important to note that all functions of the chips were validated inside the instrument.

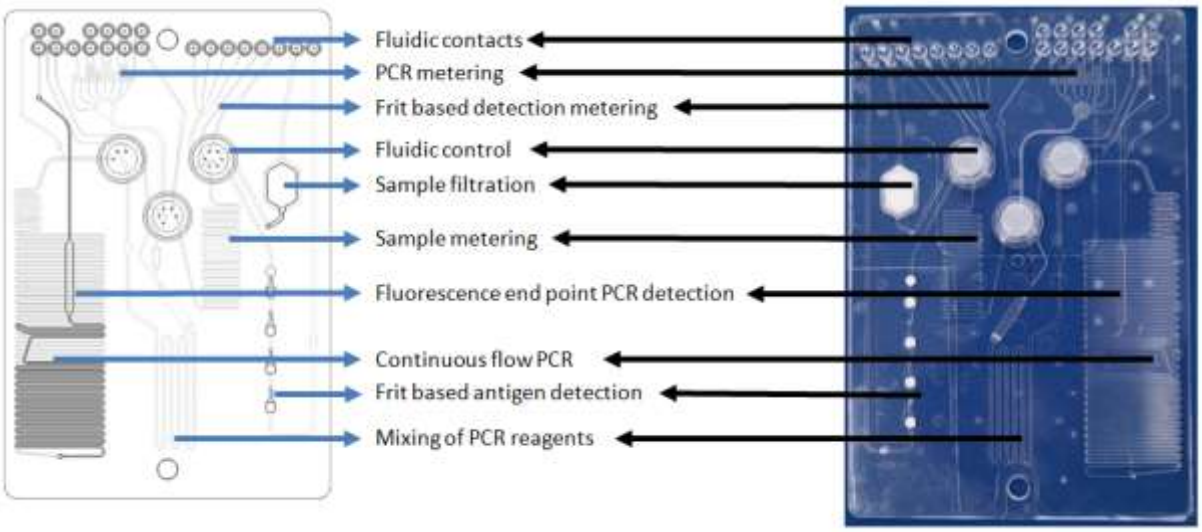


Figure 14: Illustration of the permanent monitoring chip – Multisense design 14

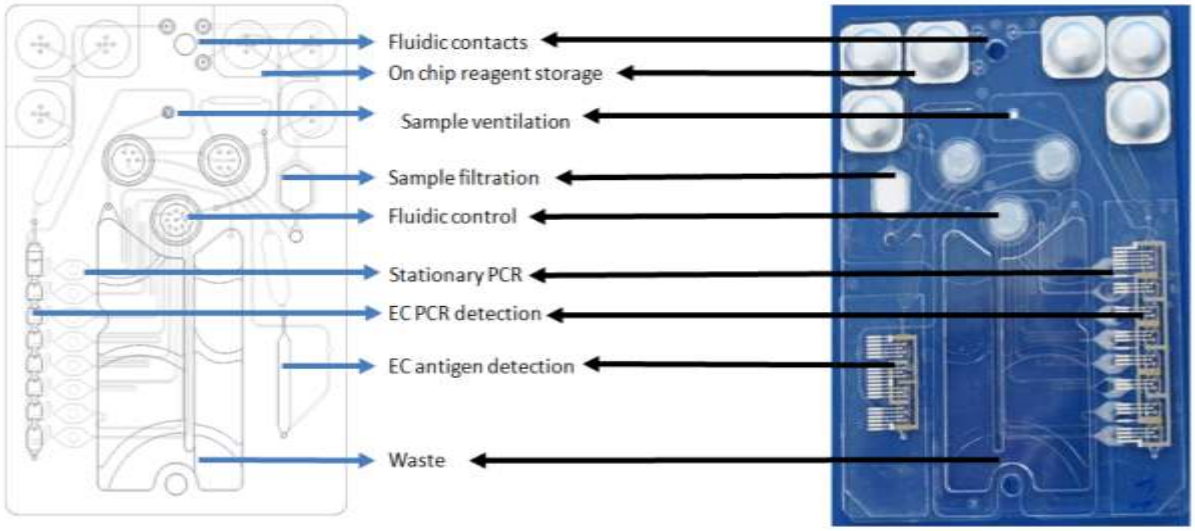


Figure 15: Illustration of the One-time-measurement chip – Multisense design 15

In brief: Special highlights are both integrated chips, the Multisense Chip design 14 (MUSE 14) for the Permanent Monitoring System and Multisense Chip design 15 (MUSE 15) for the One Time Measurement System. All biological steps were implemented successfully ending up with fluorescence and colorimetric detection for the Permanent Monitoring System and an electrochemical detection for the Permanent Monitoring System. From quick exploitation point of view, all single modules developed allow for a return on invest prior to the end of the product development phase of the integrated systems.

Summary of the S&T area MICROFLUIDICS AND MICROSYSTEM INTEGRATION WP 5 main outcome and exploitable results:

- Various microfluidic modules for PCR, sample preparation, liquid storage
- Fluidic accessories like liquid storage modules and fluidic interfaces
- Working integrated chips to cover the full process that require a further product development phase

4.1.5 S&T area SOFTWARE, COMMUNICATION & INFORMATION MANAGEMENT WP 6

The development of the communication module was also successfully achieved and implemented. Three different communication strategies namely Ethernet, GSM and satellite based communication were integrated. A major achievement was the final integration of the satellite communication and having access to such communication tool. Satellite modem proved to be easy to handle, even if not as easy as S-band device as envisaged with the first choice provider that could not be further addressed during the course of the project.

The integration and test of the ICT platform was performed successfully. The communication module proved to handle messages correctly through all communication channels planned (Ethernet, GSM, and satellite). Also, hardware and communication channel controls proved to work efficiently as well as error handling so that messages are still correctly sent, if a piece of hardware, software, or network is down. The integration on the embedded control PC and other components was solved including user interface for configuration of the communication parameters.

The embedded resulting architecture is represented in Figure 16.

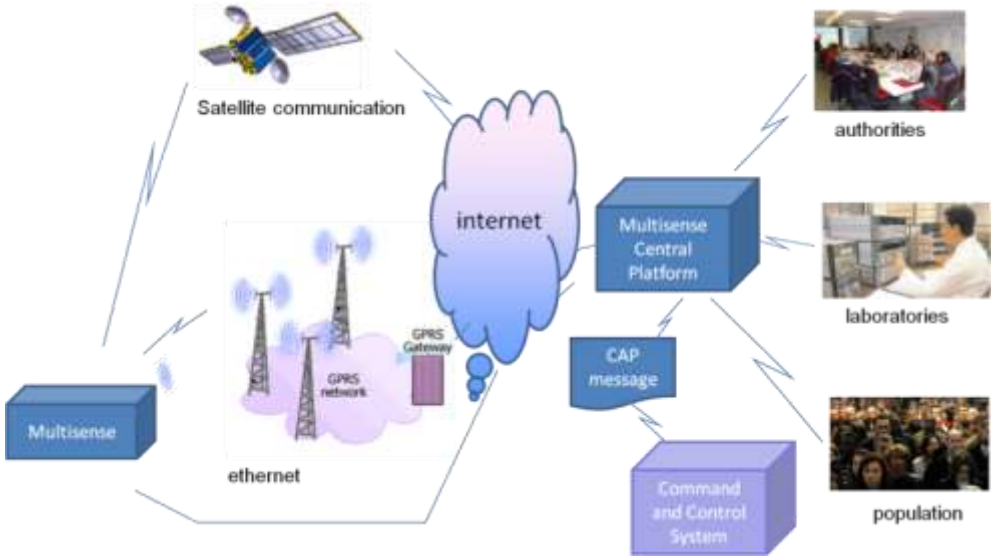


Figure 16: Multisense Chip communication architecture

Summary of the S&T area SOFTWARE, COMMUNICATION & INFORMATION MANAGEMENT WP 6 main outcome and exploitable results:

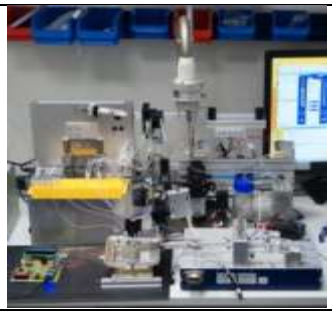
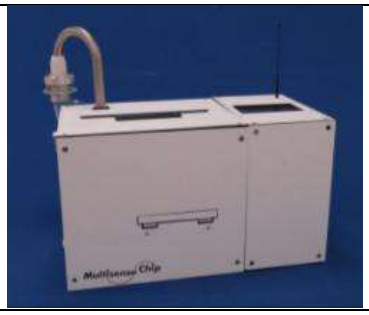
- Implementation of the communication chain with portable analytical system and addressing the command chain of disaster cases

- Accessing the satellite communication tool and integration in the communication network

4.1.6 S&T area INSTRUMENTATION AND SYSTEM INTEGRATION WP 7

Modules as building blocks covering PCR, sample preparation, sample concentration, air sampling and various detection technologies were developed, validated and the most appropriate ones were integrated in the final systems.

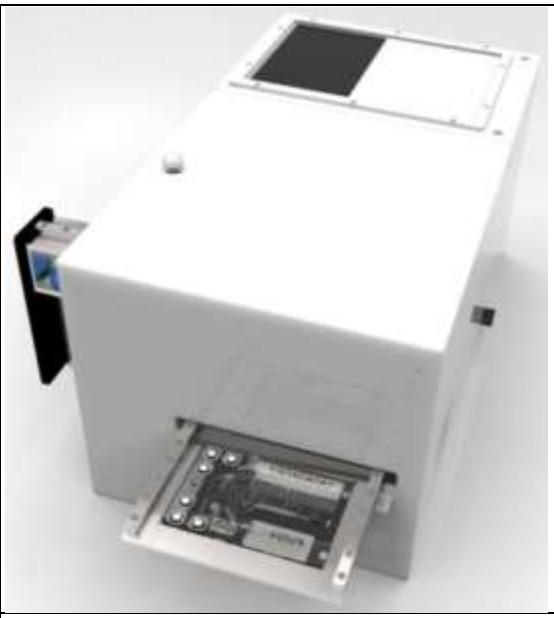
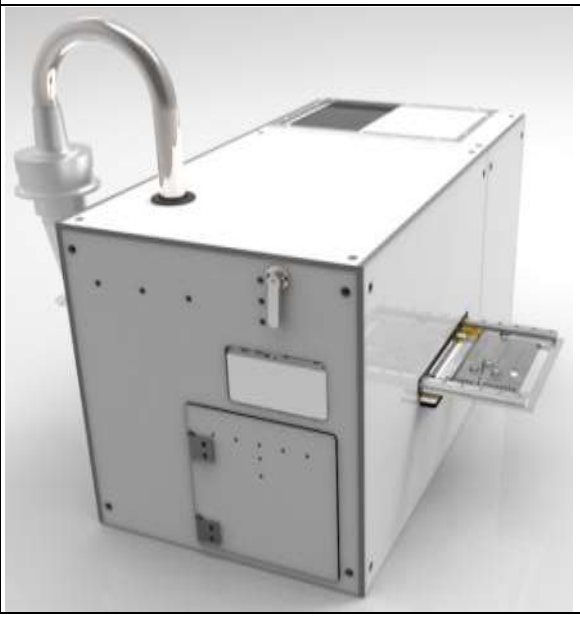
Figure 17 - Figure 22 indicate several of the building blocks used to realize the final instruments.

<table>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Figure 17: Continuous and dry air sampling tester set-up</td><td>Figure 18: Adapted bead-based sample preparation module to allow for aggregation of the beads as bands</td><td>Figure 19: Real-time Boyle-Marriotte PCR set-up</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Figure 20: Electrochemical tester set-up</td><td>Figure 21: Array and immunoassay tester set-up</td><td>Figure 22: M18 instrument: integrated immunoassay tester set-up</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Figure 23: Bench arrangement of modules of the integrated system</td><td>Figure 24: Multisense Chip Permanent Monitoring System (PMS)</td><td>Figure 25: Multisense Chip One Time Measurement System (OTM)</td></tr>
</table>

The integration of these modular building blocks beginning with the air sampling module over the EC-tester setup and two different PCR approaches was accomplished within the Permanent Monitoring System (PMS) and the One-Time-Measurement-System (OTM).

Table 1 summarizes the key performance indicators of both systems

Table 1: Key performance indicators and design sketch of the OTM and the PMS

Key performance indicators	OTM	
Power consumption	24V 10.5 A DC	
Weight	Approx 15 kg	
Communication	Results directly displayed on screen	
Control	Embedded PC with touch screen	
Depth	53 cm	
Width	37 cm	
Height	37 cm	
Detection Method	Electro chemical	
Targets DNA	8	
Targets Antibody	2	
Sampling	Swab Liquid	
Process time	approx 2h	
KPI	PMS	
Power consumption	24V 10.5 A DC	
Weight	Approx 20 kg	
Communication	GSM, LAN, Satellite Modem	
Control	Embedded PC with touch screen	
Depth	40 cm	
Width	60 cm	
Height	55cm	
Detection Method	Fluorescence, frit photometer	
Targets DNA	8	
Targets Antibody	2	
Sampling	Air sampling	
Process time	approx 4h	

The PMS for automated sampling and pathogen detection has the following key features: integrated air sampling module, cooled reagent storage, sample concentration module, continuous flow PCR module, fluorescence based DNA detection, light transmission based antigen detection, embedded PC with touch screen control, and communication module for communication via GSM, Ethernet and satellite (Figure 26).

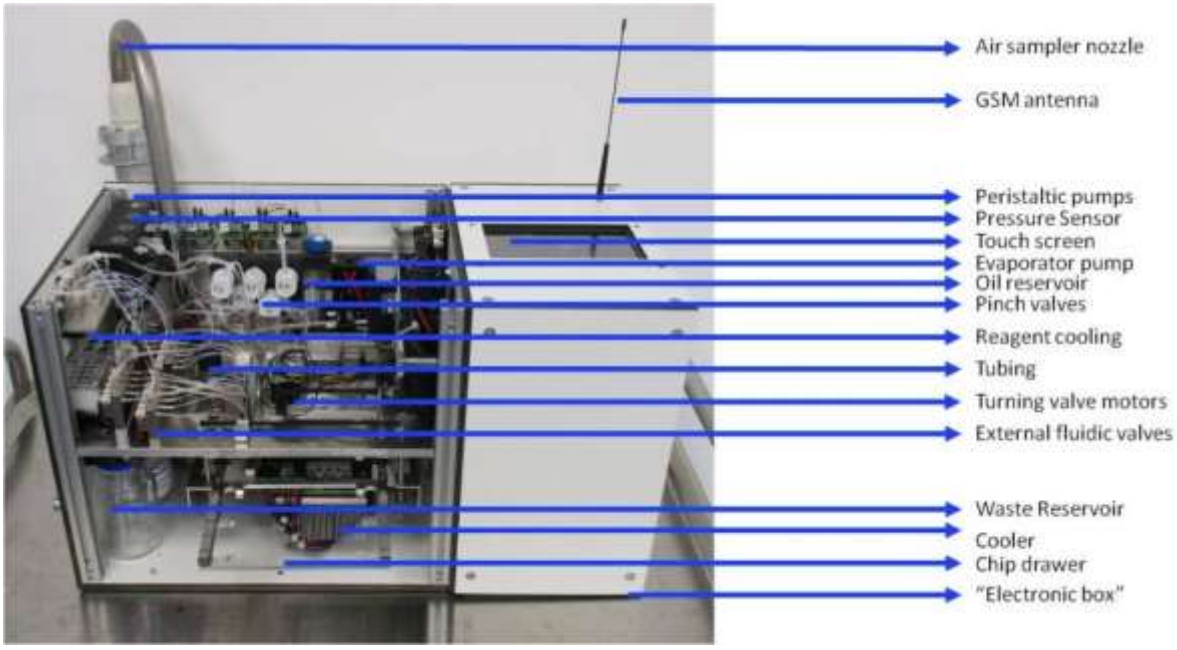


Figure 26: Detailed overview of the PMS interior

The OTM for on-site pathogen detection from liquid and swab samples has the following key features: sample concentration module, reagent storage on chip, waste management on chip, electrochemical detection of DNA and antigens, and embedded PC controlled via touch screen (Figure 27).

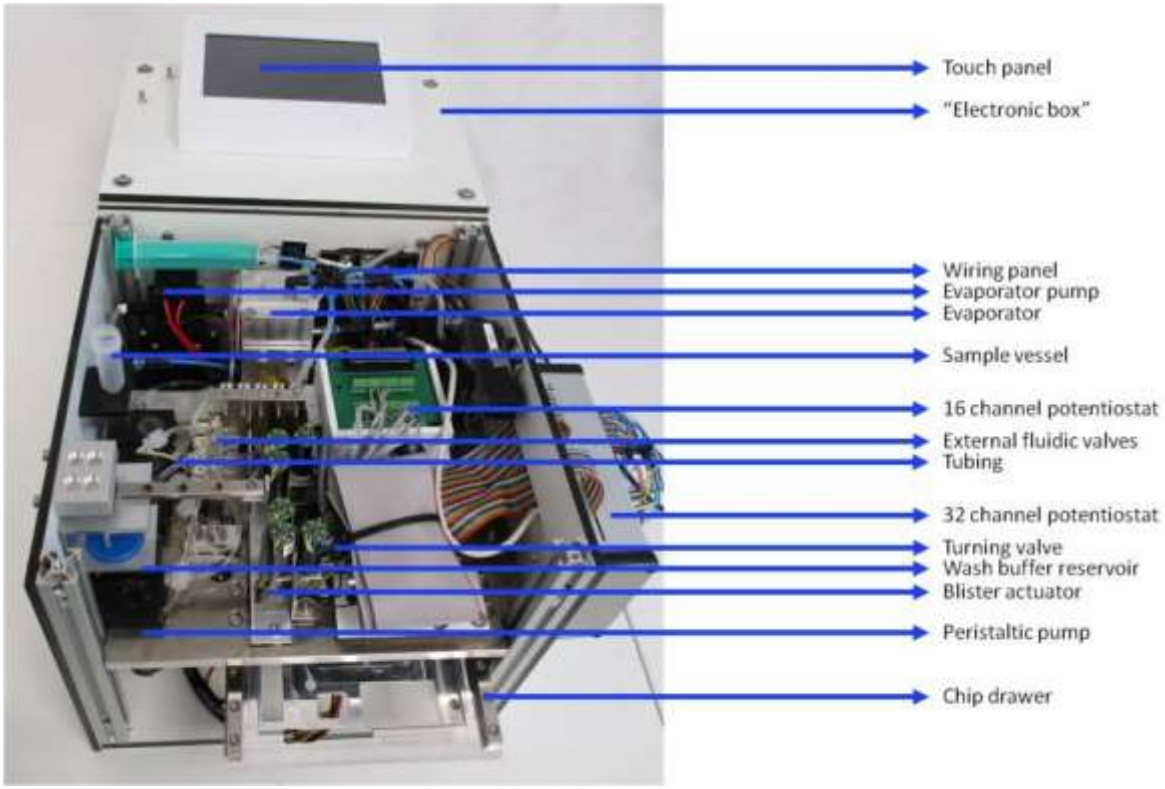


Figure 27: Overview of the OTM interior

The PMS for automated sampling and pathogen detection has the following key features: integrated air sampling module, cooled reagent storage, sample concentration module, continuous flow PCR module, fluorescence based DNA detection, light transmission based antigen detection, embedded PC with touch screen control, and communication module for communication via GSM, Ethernet and satellite.

The OTM for on-site pathogen detection from liquid and swab samples has the following key features: sample concentration module, reagent storage on chip, waste management on chip, electrochemical detection of DNA and antigens, and embedded PC controlled via touch screen.

The fluidic protocols developed for the permanent monitoring and on-time detection chips were transferred into automated control protocols for both instruments. These protocols were tested fluidically and with biological samples and reagents. Furthermore, the information transfer from the analysis software to the operator of the system has been developed and evaluated.

In brief: Both integrated instruments for One Time Measurement and Permanent Measurement were successfully realized. A wide variety of single modules were developed in the course of reaching the final goal and can be moved to the commercialization phase.

Summary of the S&T area INSTRUMENTATION AND SYSTEM INTEGRATION WP 7 main outcome and exploitable results:

- PCR lab-on-a-chip module for commercialization in 2016
- Real time PCR lab-on-a-chip module for commercialization in 2016
- Tester set-ups for electrochemical and electrochemiluminescence detection
- Available pluripotentiostat
- Electrochemical read out system
- Two integrated analytical systems (OTM & PMS) were realized that will be transferred in a product development phase

4.1.7 S&T area complete process implementation: VALIDATION, END-USER EVALUATION (WP 8) and DEMONSTRATION OF PRE-PRODUCTION PROTOTYP (WP 9)

Basis for the integration of the complete process was the validation of all single technical modules and the implemented biological procedures.

Field and end-user were integral part of the work plan: Field test with the air sampling module and demonstration of the communication technology were carried out successfully also mimicking different scenarios like human environment in sport settings or in dusty surroundings in stables. The microfluidic modules such as the PCR module for continuous flow and stationary PCR, the sample preparation module for magnetic bead-based on-chip sample preparation, the sample preparation module by thermal concentration, or the air sampling module were validated, which allowed for optimized integration of these modules in the entire lab-on-a-chip system.

Both integrated instruments, the Permanent Monitoring System and the One-time Measurement System, with their appropriately integrated microfluidic chips Multisense design 14 (MUSE 14) and Multisense design 15 (MUSE 15). Although some issues on the Multisense

design 15 (MUSE 15) were revealed during the electrochemical detection validation, it could be shown that both chips gained comparable results for reliability and reproducibility as the single modules. The overcoming of the remaining identified issues has to be a major task for the product development phase of both systems.

The validation of the physical parameters of the instruments and chips was accomplished: Robust reaction conditions are of prime importance for a reliable and reproducible detection result. It was demonstrated that the instruments in combination with the chips provide such stable conditions.

Validation of the control software and the communication module showed no issues the software and the detection protocols run reliable and were easy to use.

An end-user demonstration event took place evaluating the handiness and functionality of the Multisense Chip Permanent Monitoring System and the Multisense Chip One-Time Measurement System. End users from the “Brigade de Sapeurs-Pompiers de Paris” and the “Wehrwissenschafliches Institut für Schutztechnologien, Munster” had the opportunity to test extensively and define the list of topics to be improved for both systems.

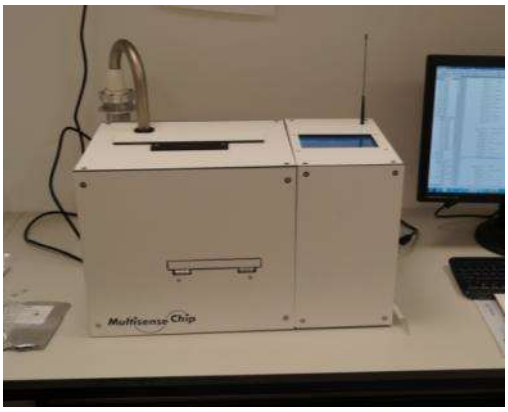


Figure 28: Permanent monitoring system

Figure 29: Demonstration of the instrument functionalities

Figure 30: End-User handling tests

Figure 31: End-user performing tests on the PMS wearing the protective gloves

Figure 32: Figure 2- One-time Measurement System

Figure 33: End-user testing the handling of the OTM consumable wearing the protective suit

Figure 34: End-user testing the handling of the OTM wearing the protective suit

Figure 35: End-user performing tests on the One-time Measurement System

It was stressed that the test results were correct, there were no false positive detections and the overall system worked.

During the tests several issues regarding performance and handiness of both instruments could be addressed transferred in the recommendation list for improvement combined with a positive feedback of the end users. The acceptance of the end users was high. And the end-users feedback has been very fruitful and will be the base for the product development phase.

4.2 Main S&T results/foregrounds – Summary

In summary, the project goal was reached resulting in two integrated system covering the application scenario of permanent and one-time measurement. The final integrated systems combine hardware, software, fluidic performance and assay implementation aspects and were successfully validated to function in a sample-to-answer manner for the analysis of bacterial pathogens on immunological and molecular level.

5 Potential impact, main dissemination activities and exploitation of results

Potential impact: The potential impact covers first of all the direct use of the developments carried out within the project for the target and other applications as well as the technologies that were established. Gathered reputation and economic results are further impact that needs to be stressed. Due to the untypically high and prominent representation of female scientists in the project, a special socio-economic can be reported.

Impact of the systems and their application

The two systems developed within the project, namely the “Multisense Chip Analyzers” for one-time and permanent measurements have a significant impact on how biological analysis, be it for the safety and security scenario, environmental and food screening of medical diagnostic can be carried out.

Due to the fact that with these systems a lab-free-approach to analyze biological samples – usually complex, time consuming, requiring lab space and trained staff – is at hand an interesting tool is at hand. Taking the **permanent monitoring system** that samples air over defined periods, this system has an impact in the security and safety area e.g. for critical infrastructure monitoring, in stadiums, metro stations, air ports or moving in the civilian application to monitor climate control units e.g. in hospitals seeking for critical and usually multi resistant bacteria.

The one-time measurement system allows to quickly analyze suspicious samples at the place of interest and can be placed in critical infrastructure, post offices, airports etc. wherever this material might come up. Expanding the use and sample matrices the medical diagnostic market has a great potential, enabling test labs as well as the respective units of hospital or even the practitioner to carry out the analysis. A future scenario is home testing. Obviously, the impact on the health market has a he impact, despite this requires a major development effort. Other application in food and environmental testing would require special developments as well but in particular contamination testing would be of major interest. Targeted development projects would quickly allow to develop solutions for this area.

Impact on safety & security & international traffic

The envisaged benefit of the project is the provision of versatile B-detection system to enforce the European safety and security actions and the tools being available.

In particular under the current threat scenario of the IS and availability of technologies to culture and distribute biological agents, the member states are urgently needing such detection systems as developed within Multisense Chip.

Taking a different user scenario into account, internationalization and upcoming epidemic illnesses like Dengue, Zika or Yellow fever virus induced ones can make use of the same technological base.

Impact of the system in a wider scope

Taking the grand challenges of an aging society into account, home testing of the health status combining the lab-on-a-chip approach with the communication tools is a future, challenging

but in particular important future impact. This obviously will require enormous effort to the ease of use, system size and cost, consumable cost and the use of smart phone in combination with health apps and communication strategies. Enabling tools were developed within the scope of Multisense Chip.

Impact of the technologies

The technologies that were developed will be included in lab-on-a-chip systems with increased performance e.g. through novel reagent implementation options, sensor integration technologies or new fluidic building blocks used to developed the integrated cartridges. Both involved SMEs (iMicroQ and MFCS) and their customers and partners will benefit from these developments.

Impact on reputation & actions deriving thereof

Due to the fact that the project brought the team members in the positions, to present their work the relevant players, on a long term view recognition of the partners was achieved. This led e.g. to the participating of MFCS in the expert group (PASAG – Protection and Security Advisory Group) of the European Commission to give advice for the follow-up Research program of Horizon 2020.

The active dissemination activities gave e.g. MFCS an awareness that the company and a system based on work carried out within Multisense Chip was shown on Germany TV celebrating the German Reunification as example of “Eastern Success Stories” showing the promised “Blühende Landschaften” (Prosperous Landscapes) from old chancellor Kohl.

Impact on the economy

The project enabled the involved industry to develop new products, build know-how and benefit from a multidisciplinary consortium. Finally, it is envisaged to achieve a double digit Mio. Euro annual turnover based on the outcome of the project. E.g. “Multisense Chip” allowed MFCS to create new business and was one of the key enabler to employ 10 more team members in the company group, partly for the project itself, but mainly to cover new topics deriving from the project’s outcome. From project application to finalization of the project, MFCS has doubled its staff also demonstrating the economic impact of the technology and the effect of EU financial support on international competitiveness. Currently two spin-off-companies are in discussion that will benefit from Multisense Chip outcome.

Impact for Europe

The benefit for Europe through Multisense Chip matures on three levels, starting with scientific excellence, over the economic impact to finally the Multisense Chip products covering the security and safety related perspective. And the achievements already made, nicely demonstrate that the funding provided was fruitfully used to achieve the promised goals.

Impact – wider societal implications

Apart from these results related to the work done in the project, this project had an additional impact as side effect. Both, project coordinator and project manager as well as other leading scientist at the partners URV and FLI were female. This is still completely unusual in a technical

field – in particular in a 'high tech' area. This project coped with an outstanding way to the still interesting item of gender issues via allowing this team not only to prove to run this project successfully but also enabled the female scientists to present the work at high level events for international audience predominated by the usual gender distribution. With the help of this project it was clearly shown on an international floor, how Europe supports women and their impact in science and business. For girls and young women, who have been addressed by the project as well e.g. via girls' days, it gives an insight in an innovative technology field and an impression how fascination for science can be turned into fun, scientific excellence, and economic success leading to more young women in science.

Furthermore, with Multisense Chip the project coordinator Dr. Claudia Gärtner was two times finalist in the Women Innovator Competition of the European Commission and Dr. Gärtner was decorated in 2014 with the Emily-Röbling-Prize for female business power.

Main dissemination activities

The complete bundle of dissemination tools was applied during Multisense Chip: Starting with the creation of an Exploitation Committee followed by the preparation of dissemination templates, the project homepage and material like flyers and posters a roadmap how to identify and address the respective players and create business from the project's outcome was defined. Major methods to create awareness were conference, congresses and trade fairs combined with scientific publications. MFCS achieved own Multisense Chip sessions at one of the major conferences of the SPIE dealing with safety and security. Working with interest groups and joining panel discussions on the safety and security topic lead to a well appreciated awareness of the projects and the coordinator MFCS as a known player in this field. More than 20 participating in relevant exhibitions and trade fairs, more than 45 oral presentations, 8 peer reviewed paper, 25 published paper, posters and activities addressing the local population demonstrate the active work in the dissemination area.

Special highlights of the dissemination events were being panelist of the event to present the "Masterplan civilian security economy" of the German Ministry of Economy and Technology, the Panel discussion on "Flagship EU-projects" during the Millipol 2013 and the EDA conference "Security Matters" 2014 (see Figure 36). Special visibility created the presence in the IB-Consulting series on NCT (Non conventional threats) CBRNE (Figure 37), that resulted in awareness and partners for distribution and promotion of the Multisense Chip results.

Figure 36: Activities at interest group events – German safety & security event (left) and EDA event on European Security – industrial panelist Airbus Defense and microfluidic ChipShop

Figure 37: Demo events by IB consultancy joined in Malaysia (left) and Washington (right)

Exploitation of results

Exploitation of results happens on four levels taking also the different kind partners into account: The first level is the creation of awareness, reputation and collaboration being relevant for all partners: This includes publications, being member of interest groups, getting awareness as player in the field, contact to distribution partners or partners for further collaborations and follow-up R&D projects. All partners have been active on this level. Also the second level “Know-how” has been exploited by all partners in respect to new technological and application know-how. “Products”, described as exploitation level three, are the most visual outcome of the project, the relevant level all industrial partners benefit from. The fourth level is the “End-user” view represented by FLI who will finally use the results.

The exploitation of results has already started, a general exploitation scope can be given and some prominent examples are highlighted.

General exploitation scope

The final results of the project were two analytical systems covering the detection of biological pathogens as a permanent monitoring system and as a one-time measurement system.

These systems channel the way to an improved, easier, and faster detection of biological pathogens and will enable responders and authorities to quickly get aware of potential contaminations and to be able to decide on potential contaminations with such agents leading to a possibility for a fast and appropriate measure. Far beyond this security driven application, veterinarian and medical diagnostics will benefit from such systems in long term.

Besides the complete system, also modules thereof such as improved air sampling system with added functionalities, and a set of microfluidic toolbox components, e.g. lab-on-a-chip consumables and instruments for sample preparation, enzyme assays, PCR etc., visualize the results of the project.

Both integrated systems and modules have the potential to impact laboratory or analytical routine. A major outcome is that in future untrained personnel can operate complex analytical tasks allowing for a new pathway to operated risk situation not only in the security but in particular to address outbreak scenarios in the veterinarian field.

The integration level provided by the communication and information aspect covered in this project will further facilitate the use of the system in critical environments and will give an automatism at hand, how analytical results will be transferred into actions, such as generating a message and starting a communication process directly by the “Multisense Chip Analyzer”.

Next to the direct products that came out of the project, their use for the partners involved in this project and the future users, the network established within this project has been of major benefit for the partners. The SMEs benefit from the interdisciplinary know-how provided the access to special infrastructure at the institutes, the experiences in hardening and validation of Bertin as larger company and their existing network, whereas Bertin gets access to a new technology and potential novel products.

Projects making use of the outcome of Multisense Chip

The created awareness through the Multisense Chip project turned into the partnering in two R&D projects within the European framework and an EDA-Contract: The FP 7 project EDEN: Achievements of the Multisense Chip fluidic design was used to create further designs and to be able to join a demo in the food area in April 2016. A Horizon 2020 project: Project to start mid 2016 were the PCR fluidic part will be complemented with a real time optical detection. A development contract for the EDA (European Defence Agency) called RAMBO combines the real time PCR to be read-out optically with a RAMAN-based detection. The project is ongoing. For this project a Bertin air sampler is in use.

Current plans for development with external partners are a Dengue and Yellow Fever platform based on work carried out within Multisense Chip.

Module – side products with direct exploitation impact

In order to realize the overall integrated system a modular approach was chosen to minimize the development risk and being able to develop single units in parallel. The outcome of this building block-work are several new products: A blister test-platform for liquid storage, a lab-on-a-chip mini-thermocycler with and without real-time detection and a novel multipotentiostat for a 64 channel. Furthermore, a novel air sampler option and sample preparation units for sample concentration and communication tools are exploitable on the short term from the companies involved.

Impact, dissemination & exploitation – summary

Due the fact that the Multisense Chip project was constantly represented at conferences, exhibitions and public events a high level of awareness for the project could be brought up

In particular the activities to address the CBRNE community were accelerated, e.g. with a participation in the CBRNE meeting in Berlin in October 2012 or presentations on the SPIE Security and Defense meeting in May 2013 in Baltimore. Further events in 2013 as the CBRNE ASIA or the Medical B-Defence conference followed. Besides the directly CBRNE focused events Multisense Chip results have been presented on numerous trade fairs partners being present.

For the exploitation in the CBRNE field, the integrated instruments are the final exploitable results. Starting with test users the return on invest will mature on a longer time frame compared to the exploitation of technologies and sub-modules that can be used for various applications.

Publications are important for exploitation and dissemination as well. Various publications were done. Besides conference presentations, several proceeding papers were published. This

included e.g. several papers and posters at the SPIE Defense, Security & Sensing or the SPIE Photonic West.

The fact that exploitation took place on different levels, ensured that all partners got a return on invest and that the risk of complete failure was minimized. In particular the SMEs profited from technologies and modules that could be exploited shortly after the project end resulting in a broader product and technology range, increased turnover, and the creation of new job.

The networking within the consortium and via contacts to further groups and networks mediated by other partners was strengthened. All involved parties were able to create new contacts and improve already existing ones for future collaboration on R&D as well as commercial level.

New R&D projects and contacts to distributors are the direct outcome initiated through the Multisense Chip project that will be a base for the commercialization of the integrated Multisense Chip products.