SNIFFER

Sensory devices network for food supply chain security

Executive Summary

The presence of such CBR agents in the food supply chain can lead to adverse health effects in humans and animals as well as great economical losses. Furthermore, it exhibits multiple vulnerable points. A very relevant 20% of all deliberate incidents recorded between 1950 and 2008 (as identified by the SECUFOOD project – Security of European Food supply chain) took place in Europe. Further analyses by SECUFOOD have shown a growing trend both in the number of incidents and in the number of casualties resulting from those incidents. Control activities require suitable tools in order to provide the required analyses in a timely and highly qualitative fashion. Flexibility to adjust to changing threat scenarios is also of great interest. Present-day analysis schemes, which consist of sampling, transportation to a laboratory and often tedious analytical methods leave room for improvement. The SNIFFER system aimed to improve this situation through the

- Development of a portable sensor device which is easy to use with fast detection times and with an easy decontamination/cleaning procedure based on Molecular Imprinted Polymer (MIPs) technology.
- Development of novel fluorescent and colorimetric probes that can be used in the labelled detection of toxins, enzymes/proteins and other microbial features and augment the capabilities of MIPs technology.
- Development of modular connectivity enhancing components that can be applied both to the SNIFFER sensor device as well as to existing commercial devices. These enable COTS sensors and the SNIFFER sensors to operate in networked fashion.
- Development of a database of the most dangerous and heritable pathogens and toxins which may be found within the food supply chain and which may affect large populations.
- Design of a network of sensors in which each sensor transmits its information to a command centre, from which the user may issue commands to the sensors and monitor the SNIFFER system.
- Validate the proposed sensor and platform in the laboratory and in an environment that simulates a particular scenario (and to compare it to existing detection methods)

Throughout its duration SNIFFER achieved the following:

- A set of functional and operational requirements for a CBR detector in the food supply chain was created
- A set of scenarios for the application of CBR sensors in the food supply chain was proposed.

- A system architecture for the SNIFFER system comprising COTS sensors and MIPs and fluorescent probe sensors located at any point in the supply chain and interconnected to a command canter was proposed.
- A set of guidelines for the use of COTS and MIPs based sensors for the detection of CBR agents including two user guidebooks were developed
- A survey of existing sensors appropriate for use in the food supply chain was carried out
- A set of procedures for food sample preparation (including packaging and transport) for analysis with COTS or MIPs based sensors was created.
- Development of TRL5/6 functional prototypes of potentiometric sensors based on MIPs for the detection of pyrrolizidine alkaloids, bacillus cereus and cereulide, quaternary ammonium compounds (QAC) and bacillus anthracis
- Development of a library of luminescent and colorimetric probes at TRL5 for 3 target agents (bacillus cereus and cereulide, methylmercury and explosive TATP in vapour phase)
- Development of a SNIFFER connection device at TRL7 providing physical and logical interface for existing sensors and devices, networking capability over Ethernet, wireless local area network (WLAN) and cellular networks, computing power to carry out data processing and data fusion algorithms and a software agent for managing data collection and transfer from sensors to a back-office server
- Development of a SNIFFER command center interface at TRL7 comprising multiple functionalities
- A set of general recommendations on how authorities could adopt SNIFFER like technology and systems and three policy briefs were proposed
- Validation of the SNIFFER system for the dairy production chain. This included an analytical performance validation part as well as operational trials, and demonstrated the capabilities and good performance of the developed system as well as the fulfilment of various requirements, while at the same time highlighting areas for future improvement and development.

Summary description of project context and objectives

The continuous growth of world population is accompanied by higher demands of food products. Agriculture is forced to apply intensive production methods, capable to support the growing needs in raw materials required by the food industries. In addition to being plentiful, food products should be safe and cover nutritional requirements. The efforts of satisfying the increasing demands on safe food products are, however, threatened by potential contaminations of various nature (chemical, biological or radiological; i.e. CBR agents). The presence of such CBR agents in the food supply chain can lead to adverse health effects in humans and animals as well as great economical losses. World-wide trade of food products brings with it the additional danger of rapidly distributing contaminated foods to many countries and populations.

In the European Union, the responsibility for placing safe foods on the market rests with the food business operators (producers, processors, distributors, wholesalers and retailers). They are required to have appropriate quality assurance systems in place and to assess any potential hazardsby HACCP (hazard analysis and critical control points) principles. Beside these self monitoring systems, official controls are carried out according to regulation (EC) 882/2004. The implementation of risk based control plans forms a part of those efforts.

Both control activities require suitable tools in order to provide the required analyses in a timely and highly qualitative fashion. Flexibility to adjust to changing threat scenarios is also of great interest. Present-day analysis schemes, which consist of sampling, transportation to a laboratory and often tedious analytical methods leave room for improvement. The SNIFFER system was proposed and developed to contribute to improving this situation.

Project SNIFFER had the clear objective of rendering the food supply chain more secure by applying both commercially available devices and novel technology (namely Molecular Imprinted Polymers - MIPs based sensor technology) in a networked environment to the detection of CBR agents in the food supply chain. The network can cover the entire food supply chain if need be, using several detection devices spread across more vulnerable points (such as cow grazing, milk farm, cheese factory, transport hubs, etc…) of the food supply chain. This goal was detailed into several lower level objectives:

- Development of a portable sensor device which was easy to use with fast detection times and with an easy decontamination/cleaning procedure.
- Development of novel fluorescent and colorimetric probes that could be used in the labelled detection of toxins, enzymes/proteins and other microbial features and augment the capabilities of MIPs technology.
- Develop modular components that could be applied both in the SNIFFER sensor device so that each of the components could be replaced independently for either maintenance or upgrade purposes, and in existing commercial devices to support interconnectivity.

- Develop a database of the most dangerous and heritable pathogens and toxins which could be found within the food supply chain and which could affect large populations, in order to ascertain which agents the SNIFFER system should detect. Develop a contingency and countermeasure plan to address an event of a positive CBR agent detection.
- Design a network of sensors in which each sensor transmits its information to a command centre, from which the user can issue commands to the sensors and monitor the SNIFFER system.
- Validate the proposed sensor and platform in the laboratory and in an environment that simulates a particular scenario (and compare it to existing detection methods)

The SNIFFER system comprised components for sampling (no specific development work was done in this area) and sample preparation, the MIPs and COTS sensors for the actual analysis of CBR agents and finally a connection device to which the sensors were attached and which transmitted the collected data to a command center as depicted in **Figure 1**.

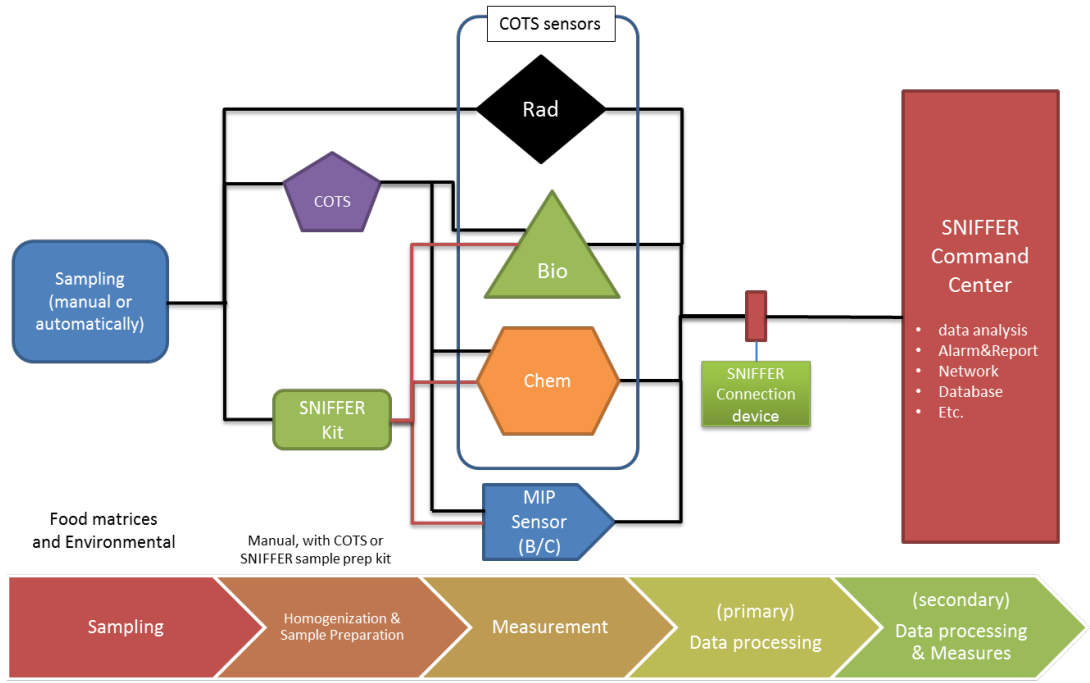

Figure 1: SNIFFER Concept

Description of main S & T results/foregrounds

SNIFFER began its work with the development of scenarios for the application of the system and a strategic selection of target CBR agents. Known incidents of natural, accidental and deliberate contamination within the food-chain were compiled in a database and used as basis for a number of strategic scenarios. This was further used to select a validation scenario. A list of CBR agents was established to select the most relevant ones for SNIFFER. A literature review of previous intentional and accidental incidents was developed to identify the most vulnerable stages and relevant agents along the food supply chain. Focus was placed on incidents where many people were affected and/or cross border economic impacts were significant.

SNIFFER established a set of functional and operational requirements in 3 domains:

- Requirements for sensors and network system development (including command center, connection device, MIPs sensor for biologic targets and MIPs sensor for chemical targets);
- Selection criteria for COTS sensor integration (biological, chemical and radiological sensors);
- Sample preparation.

In the first domain 52 requirements were derived. Concerning COTS integration 45 relevant criteria were identified. Finally, 20 requirements were defined for sample preparation.

A high-level system architecture considering the following aspects was proposed:

- Framework of the system explaining layers, functional modules and operations
- High-Level flows defining data, command and control flows between functional modules
- Core components and interfaces explaining choices for the different components
- Command Center architecture
- Software components
- Sensor data format and network security analysis

The project also developed the MIPs sensor tehcnology. SNIFFER combines MIPs technology with fluorescent or colorimetric indicators thereby raising reliability. The MIPs and fluorescent probes prototype sensors were successfully completed, assembled and tested.

Sensors for selected toxins were prepared by Molecularly Imprinted Polymer (MIP) technology. In the case of silica-based MIPs the monomers to be used in the preparation of sensors were chosen according to the possibility of having relatively strong, non-covalent intermolecular interactions with the functional groups present in the analyte (eg, links via hydrogen bonds). Two types of polymerization were studied: the conventional polymerization and electropolymerization. In the first case, the polymer precursors were applied by screen-printing on the substrate; in the second case the passage of an electric current promotes direct fixing on the substrate. Dual-fibre optic probes for additional fluorescence detection using MIPs were designed for selective sensing without false positives. The optical fibre probe designed was coated with the MIP to select the analyte of choice and the fluorescent reporter was evaluated with respect to fluorescence sensitivity enhancements in the presence of the specific analyte. The selectivity and sensitivity of the MIP probe toward the requisite analyte was therefore improved.

The devices were tested for six chosen targets.

For target 1 (Pyrrolizidine alkaloids): Inexpensive sensors for detection of bacterial threats on food based on Arduino boards were prepared. Pyrrolizydine alkaloids were quantified spectrometrically and potentiometrically, the first technique was based on a new concept: e-eye, following e-noses and e-tongues, the determination of these compounds was performed by taking in account the different basicity among ammonium compounds.

For target 2 (Bacillus Cereus and Cereulide): Surface Imprinting Polymers (SIP) electrodes were fabricated, having the whole bacteria of three Bacillus species as template. Firstly, we worked with the safe bacteria and then with B. cereus. Four different sensors were made, each one differing of the others in the monomer composition, in this way an e-tongue was made. The use of e-tongue diminishes the probability of false positives and negatives, in this way, several electrodes for B. cereus were prepared. Cereulide was determined optically and potentiometrically, by preparing a sensitive silica probe, which in conjunction with a fixation procedure involving glycydopropylsilane and valyomycin, made possible to fabricate a fluorescent film capable to determine, indirectly, the levels of cereulide when K^+ ion was present.

For target 3 (Quaternary Ammonium Compounds): Quaternary Ammonium Compounds were determined potentiometrically using MIPs, the determination level was excellent and well correlated with the test solutions.

For target 4 (Bacillus anthracis): A sensitive sensor for the preliminary trial of anthrax specimen was executed.

For target 5 (methylmercury): a new fluorescent polymer capable to detect the presence of mercury in fish samples was developed. The modified polymer emits blue light when irradiated with UV proportionally to the quantity of mercury, as $MeHg^+$ or Hg^{2+} cations, presented in the fish. The quantitative relation between the concentration of mercury in fish and the increasing of fluorescence in the polymer in contact with fish has been confirmed.

For target 6 (triacetone triperoxide vapours): a fluorogenic material that is able to generate fluorescence in the presence of vapour of triacetone triperoxide, TATP, a home-

made explosive used in terrorist attacks, for which there was no good detection method until now, was developed. The fluorescent response given by the material to the presence of TATP is permanent so it can be checked at any time after the TATP action.

A preliminary list of 9 COTS sensors for use with the MIPs sensors to validate the overall system was produced from the analysis of more than 40 choices. The criteria used for the selection of COTS was aimed at narrowing down a wide field of products to a few that were capable of detecting three or more of the SNIFFER relevant selected chemical agents, pathogens and/or radiological contamination agents within a short time frame (less than a day). This selection process was aimed at refining the list to those that had the greatest benefits to SNIFFER. Had the selection process focused on other chemical or biological agents or a different timeframe for the reporting of analysis results then the final COTS list may have prioritised differently. Despite this restrictive selection process 24 COTS went on to subsequent trade-off analysis to identify which to include in the SNIFFER network. This methodology could also be applied by stakeholders to identify COTS of relevance to their food supply chain by including relevant food safety pathogens in addition to the CBR agents that SNIFFER has focused on. The consortium also identified 31 on-going EU projects as having the potential for knowledge transfer, collaboration and sustainability for SNIFFER. 6 of these projects stood out and 3 have been formally contacted. Plans for collaboration and exchange of information have been drawn up. General procedure principles for MIP food sensors sample preparation and handling were settled and are to be tested in WP3. Here, it should be noted that SNIFFER has not performed any work on the development of actual sampling technologies or techniques (in line with what was foreseen in the contract) but rather on the procedures to be followed during the sampling process. For the SNIFFER validation scenarios, preparation of samples will be performed by chemical extraction and clean-up (C agents) or immunomagnetic separation (B agents) with or without pre-enrichment.

Concerning the development of the SNIFFER connection device, the consortium tailored and adapted an existing TEKEVER device to support the SNIFFER requirements and specifications. The SNIFFER connection device is a network module that is flexible and can provides multiple functionalities. The connection device, as the communication module in charge of collecting and delivering data to the command centre, can be deployed in multiple options using the available communication interfaces. The connection device can be deployed using Ethernet, wireless local network and cellular networks. Three methods for data collection from sensors were considered and two of them were developed (as they require the use of the SNIFFER connection device). Tests concerning the data formats and polling of sensors as well as extraction of data from the outputs of the COTS sensors were carried out.

In order for a user to be able to interact with the sensors, the connection device and the command center functionalities, the team designed and developed the SNIFFER user interfaces. These comprised three different types of interfaces, each with its own set of functionalities associated:

- The SNIFFER command centre graphical user interface (see **Figure 3**).
- The software agent GUI version of the application.
- Dedicated user interfaces to control the different sensors considered during the project, namely: miniVIDAS, RAPID, MIPS and ELISA.

The validation of the SNIFFER system in a simulated operational scenario was developed in three different facilities: LBDB, AGES and INESC. The dairy supply chain was selected as model system. Milk and milk derived products constitute large-volume food products in Europe and consumer sensitivity regarding safety and quality of this product group is extremely high. Milk and milk products are food products of special interest due to their large consumption amounts, important role in infant nutrition and specific consumer perception. Milk and derived products undergo different processing steps to obtain the final food. CBR agents are easily introduced into the dairy supply chain from farm to ready to eat food possibly affecting human health. The simulated operational trials were:

1) miniVIDAS with PC without Connection Device;
2) miniVIDAS with Connection Device without PC;
3) RAPID with PC and Connection Device;
4) ELISA with PC without Connection Device;
5) MIPS with SNIFFER hardware protoype.

Trial # 1: *Salmonella* was detected in the milk sample and the positive result was marked in red colour in the command centre software and the location of the analysis was shown on a map in the command centre software. The communication with the command centre was successful. The positive result was registered at the command centre.

Trial #2: *Listeria* was detected in the milk sample and the positive result was marked in red colour in the command centre software and the location of the analysis was shown on a map in the command centre software. This trial used and tested a connection device to collect data from the sensor and transmit it to the command centre. The communication with the command centre and the depiction of the results was successful.

Trial #3: *Listeria* was detected in the milk sample and the positive result was marked in red colour in the command centre software and the location of the analysis was shown on a map in the command centre software. Contrary to expectation, *Salmonella* was detected as negative by the RAPID sensor. This trial used and tested a PC to collect data from the sensor and a connection device to transmit the data to the command centre. The communication with the command centre was successful and positive results were identified.

Trial #4: The ELISA sensor results were transmitted and processed successfully by the command centre. Results above the maximum permissible level were not identified by the SNIFFER software. Further adaptations were identified and will need to be carried out post-project.

Trial #5: The MIPs sensor results were transmitted and processed successfully by the command centre. Further work on processing of the MIPs sensor raw data was identified.

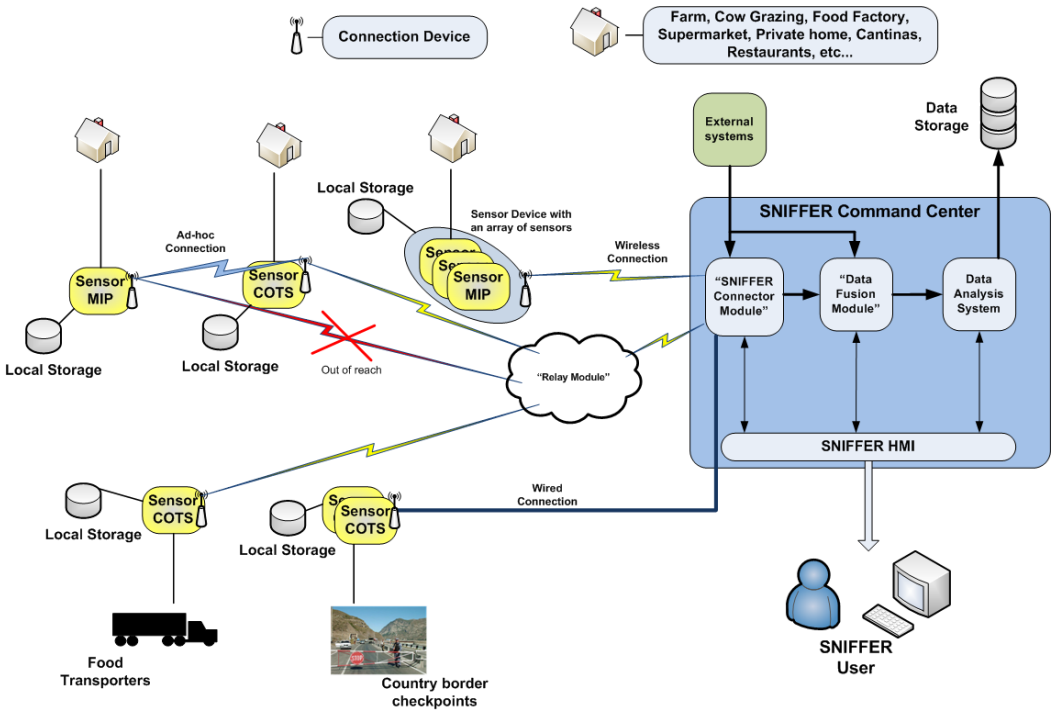

Figure 2: SNIFFER High-level Architecture Instance

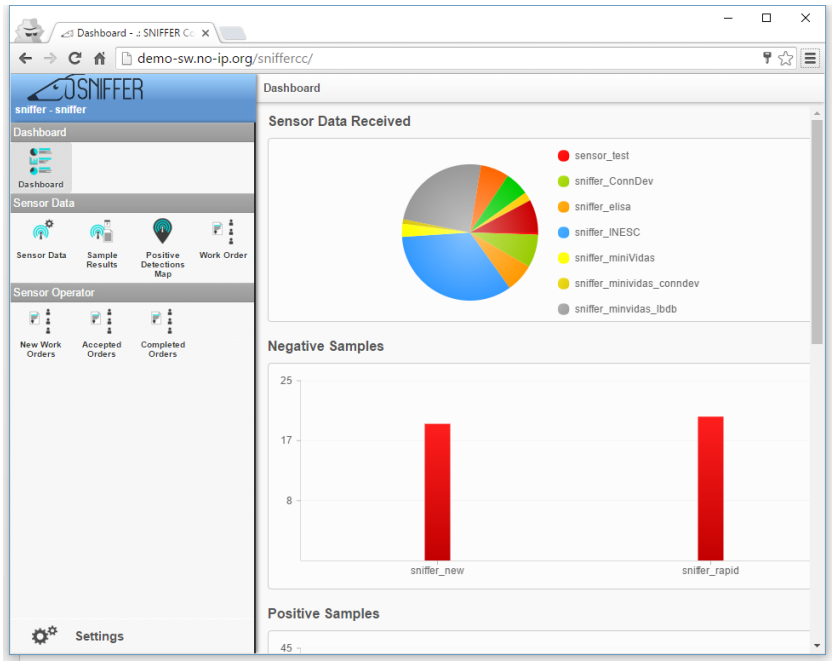

Figure 3: SNIFFER Command Centre dashboard charts

Potential impact and main dissemination activities and exploitation results

The SNIFFER final results can be summarized as follows:

- TRL5/6 functional prototypes of potentiometric sensors based on MIPs for the detection of pyrrolizidine alkaloids, bacillus cereus and cereulide, quaternary ammonium compounds (QAC) and bacillus anthracis
- A library of luminescent and colorimetric probes at TRL5 for 3 target agents (bacillus cereus and cereulide, methylmercury and explosive TATP in vapour phase)
- SNIFFER connection device at TRL7 providing
    - Physical and logical interface for existing sensors and devices
    - Networking capability over Ethernet, wireless local area network (WLAN) and cellular networks
    - Computing power to carry out data processing and data fusion algorithms
    - Software agent for managing data collection and transfer from sensors to a back-office server
- SNIFFER command center interface at TRL7 comprising
    - Authenticated user access
    - Dashboard
    - Sensor Data module
    - Sensor Operator module
- Set of general guidelines for the use of COTS and MIPs based sensors for the detection of CBR agents including the proposal of two user guidebooks
- Set of procedures for food sample preparation (including packaging and transport) for analysis with COTS or MIPs based sensors
- Set of general recommendations on how authorities could adopt SNIFFER like technology and systems
- Body of scientific publications explaining the work performed and results achieved

Concerning the limitations of MIPs and their maturity (e.g. if they are mature enough to be applied in the field or only in the lab), the following can be considered. MIPs are of limited utility on whole bacteria, because the pores must to be very large to allow bacterial translation until the recognition sites. Instead SNIFFER used a similar technique: Surface Imprinted Electrodes. This method uses normal gelatine to immobilize the bacteria or spore and then attach them to a given surface.

It is the intention of the partners involved to progress that work in order to submit a patent. SNIFFER partners have published several papers on the detection of other bacteria in water and quality control in anaerobic reactors (Jesus et al., 2009; Queiros et al., 2010; Queirós et al., 2011, 2010), MIPs have demonstrate ability to accomplish such tasks, so the feasibility of using the technology for other application domains has been achieved already.

At the conclusion of the project, SNIFFER's work is mainly at the lab stage. However, as part of the logic of the development of the technology, some of these sensors, and similar ones, will be used in a large scale project which is starting now at INESC-TEC in order to develop a fully integrated system for sea and aquaculture quality control. This project includes the production of MIP sensors and its placement in a submarine robot and in real aquaculture explorations. Both systems need to be able to make continuous measurements. In summary, the fielding of sensors based on MIPs technology is expected to take place in the short term (1-2 years).

The successful outcome for the dairy production chain allows the conclusion that the SNIFFER system may also be successfully applied to other food supply chains such as the meat production chain or the cereal production chain. The developed SNIFFER system provides reliable networking and data transmission capabilities to transmit the results of sensor analysis to the command centre either by means of a software tool or a connection device. Future improvements include the better adaptation of the employed MIP and COTS sensors to the requirements of the food supply chain. In addition, a required extension of the SNIFFER system's capabilities includes the consideration of legal limits for food products which are e.g. laid down in regulation (EC) 1881/2006 or regulation (EC) 2073/2005 in the decision process of the command centre. In that respect it needs to be considered that analyses for B agents generally obtain a positive or negative result, while the analyses of C or R agents give a numerical value. The implementation of automatically evaluated decision levels which then lead to a positive/negative result also for numerical analytical results seems highly desirable and worthwhile.

Overall, the validation of the SNIFFER system, which included an analytical performance validation part as well as operational trials, demonstrated the capabilities and good performance of the developed system as well as the fulfilment of various requirements, while at the same time highlighting areas for future improvement and development.

In terms of dissemination, the consortium was pretty active having published 6 peer-reviewed papers. An additional six are in the process of revision or being prepared for submission for publication. The project's concept, its work and results were presented at 20 national and international conferences (in some cases multiple presentations were made at the same conference). Furthermore, the project was presented to other relevant FP7 projects, was represented at two exhibitions, a flyer was created and at least one PhD thesis was developed under the scope of the project.

Concerning exploitation, the consortium identified all possible exploitable project results, surveyed the CBR detector market for potential competitor products to SNIFFER and described in better detail the potential markets. Models for the exploitation of SNIFFER results were identified and discussed. These included options such as licensing of SNIFFER technologies to project partners or entities outside of the consortium, integration of larger supply chains (namely of large system integrators) through the sale of components based on SNIFFER technology and direct sale of complete SNIFFER systems. Value propositions were developed based on SNIFFER results and several business model proposals were created. Three SNIFFER based products or services were considered:

- Fully integrated SNIFFER system (exploitation to be led by TEK);
- SNIFFER sensor component (exploitation to be led by INESC);
- SNIFFER connection device (exploitation to be led by TEK);

SNIFFER also registered with the technology demonstrator project EDEN and expressed interest in participating in one of the planned demonstrations although at the end this was not possible as the relevant demo coincided with the end of the SNIFFER project. In addition two patents related to the work performed on the fluorescent probes have been submitted.

SNIFFER presents several advantages:

- Combination of MIPs functional prototypes with luminescent and colorimetric probes reduces false positives for specific agents
- SNIFFER connection device enables instantaneous exchange of sensor data between sensors in the field (both COTS and SNIFFER developed) and laboratories. Also enables exchange of data between authorities in different countries
- MIPs sensors and probes prototypes are in a size compatible with a mobile hand-held device
- The way of working of the MIPs sensor allows for faster turn-around time between measurements in comparison with existing sensors
- The way of working of the MIPs sensor reduces the consumption of reagents and consumables
- SNIFFER MIPs prototypes and luminescent and colorimetric probes detect relevant agents as defined in scenarios of WP2 with the help of users
- Sample preparation guidelines and sensor user guidebooks improve usability of both COTS and SNIFFER sensors
- SNIFFER connection device can work with sets of existing sensors. This provides part of the capabilities of SNIFFER (namely possible reduction of reaction times and facilitates cross-border cooperation) without the need to invest in a completely new sensor system (i.e. customers can continue to use their existing sensors but now with added functionality)
- Deployment of SNIFFER connection devices and MIPs sensors along with luminescent probes across the food supply chain is feasible and improves traceability of contaminants

However, as with any system, it also has some disadvantages:

- The limitations of the MIPs technology prevent the use of the SNIFFER developed sensor in the detection of bacteria (this does not prevent the use of the networking device with a COTS sensor for bacteria detection).
- The integration of the SNIFFER system is limited by the openness of existing COTS sensors to allow connection to the connectivity device.
- The SNIFFER sensor component is still not mature enough to field – it is still necessary to improve its robustness and performance
- Packaging solutions to integrate MIPs, fluorescent probes and the connectivity modules of SNIFFER have not been developed and this is fundamental for the commercial exploitation of SNIFFER
- Sensors are not physically integrated with the connection device
- SNIFFER has not been deployed in real operational environments for extended periods of time and this may raise a confidence issue from potential customers.

The consortium believes the project has had impact on the following:

- Capability to reduce false positive rates and improve sensor robustness through the combination of novel small-molecule “switchable” fluorescent probes with the MIPs sensor prototypes.
- Capability to network different sensors at different stages/locations of the food supply chain (vertical and horizontal dimensions) reduces reaction time and improves efficiency of detection.
- Capability to improve the quality of detection through data fusion.
- Optimization of standard operating procedures and adoption of common or similar plans and procedures based on SNIFFER guidelines will reduce significantly incident response times and will raise confidence of results obtained cross-border.
- Set of policy briefs will help policy makers as well as public authorities responsible for food supply monitoring understand the benefits of SNIFFER in particular and the need for continued investments in the area of food safety (with respect to accidental or intentional contamination) in general.
- The SNIFFER validation methodology - The validation of the proposed sensor and platform in laboratory and in an environment that simulates a particular scenario (comparing the results to existing detection methods) will prove to be an invaluable contribution for the process of rendering the system field applicable, since these lessons can be used to create best practices and provide insights on how to pursue certification and fielding.

Address of project public website and other relevant contact details

More information on the SNIFFER project and the results of the work performed (including deliverables) can be found at its website at www.fp7-sniffer.eu

Partner contacts are also provided:

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