SALIANT *Collaborative Project*

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COOPERATION

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Publishable Summary

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1. Executive Summary

SALIANT (Selective Antibodies Limited Immuno Assay Novel Technology) is a Security Research project funded under the EU 7th Framework Programme for Research to develop a hand held prototype device for rapid, real time analysis of trace levels of explosives, TNT, HMX, RDX and PETN. The simple design is easy to operate and is based upon a highly innovative positive detection mechanism for small molecules incorporated into the lateral flow immunoassay format commonly found in biomedical applications.

The EU consortium consisting of an R&D group of 5 science and technology partners from academic and commercial organisations and an end user group consisting of 2 government forensic laboratories and a team of first responders investigated sampling and detection methods, technology integration and demonstration of the device prototype in both forensic laboratories and first responder scenarios. End users were involved from the beginning of the R&D process to advise on device design and performance to drive efficiency in the development process

The major outputs of SALIANT are a simple sampling procedure and a rapid and clear test suitable for use in post blast emergency situations and usable by first responders in protective equipment. The test produces a single control line, to indicate that the test has functioned correctly and a second positive test line in the presence of explosive. A hand held reader to display and store the result of a single test was also developed and successfully used in field trials. The lateral flow test, sampling procedure and reader have been designed to operate as an integrated system and can be manufactured at scale.

Prototype SALIANT devices were tested in field trials by first responders who confirmed that the current sampling protocol and reader behave well and are usable under field conditions

SALIANT also made significant progress in researching novel methods for sampling explosives in air mixtures as well as the detection of multiple explosives - developments that can be built upon after project completion.

Research results generated throughout the project were communicated to a wide and varied target audience ranging from the general public to security networks. A variety of communication tools were used depending on the message and the intended audience and included attendance at trade exhibitions and conferences, publications, press releases , and demonstration workshops. A video presentation introducing the technology and its applications has also been prepared.

The SALIANT system is intended for launch into the Explosives Trace Detection (ETD) market currently undergoing accelerated global growth at a rate of CAGR 14%. An initial competitor analysis has confirmed the uniqueness of the product as well as highlighting features which could make it attractive for many applications. Anticipated additional applications at this stage are likely to be found in screening and detection of trace explosives in the transport and military sector as well as routine monitoring for critical infrastructure and personnel.

2. Summary Description of Project

2.1 Scope of report

This report is a non – confidential summary describing the SALIANT project. The project was carried out under the Security Theme of the 7th Framework Programme for Research - Investing into Security Research for the Benefit of European Citizens. The report sets out the project's main aims and objectives and provides details of the participating organisations and their contributions made. The main science and technology results are described however due to the sensitive nature of the research care has been taken to protect certain information produced according to EU guidelines for Security Research.

The report also describes marketing information generated and the dissemination activities carried out throughout the lifetime of the project to communicate the projects aims and achievements to a variety of target audiences. This is placed in the context of the socio-economic impact that the project could offer in the future.

In the final section the report describes plans for the exploitation of the project results including potential markets for a future SALIANT system with suggestions of how the SALIANT system may find application in the short, medium and longer term.

2.2 Background to report

EU Security Research

Upholding the values of justice, freedom and security is an important objective for the EU that has to be addressed against the increasingly complex security challenges being faced today. In response to this challenge the European Security Research Innovation Forum (ESRIF) was formed in 2007 to address the complex area of security research. One of the aims of the Forum was to capture the multidimensional aspects and operational expertise of security that exists in Europe and use this to rationalise and inform decision making at the industrial, national and European level. As a result the European Security Research Innovation Agenda (ESRIA) was published in 2009 and included two important recommendations:-

- Promotion of innovation as the foundation for a European security market
- Enhanced co-ordination between institutions involved in security research and innovation

To promote innovation in the sector ESRIF outlined a roadmap for security research to be carried out under the Framework Programme for Research (FP7). To support the development of new technologies EUR 1.4 billion has been invested into Security Research under the Seventh Framework Programme for Research (FP7) from 2007-2013. The Programme was divided into security themes with the following objectives:-

- To develop technologies and knowledge needed to ensure the security of citizens from threats such as terrorism and (organised) crime,
- To ensure optimal and concerted use of available and evolving technologies to the benefit of civil European security;

- To stimulate the cooperation between providers and end users for civil security solutions; improving the competitiveness of the European security industry

In a response to the ESRIF report published in 2009 the EU Commission supported its findings and underlined the importance of the following that they recommended should be taken into account when developing all future security projects:-

- Involvement of end users at an early stage in the R&D programs to define technology specifications
- Adopt an integrative approach when developing new technologies by considering pan –European capabilities, interoperability etc
- Engage with end users at an early stage to test prototype models and gain feedback

2.3 Description of Project

SALIANT (Selective Antibodies Limited Immuno-Assay Novel Technology) was an FP7 Security Research Project funded to develop a hand held device for real time analysis of trace levels of explosives. The device design and simple operation makes it ideal for use by First Responders at crime scenes and terrorist incidents.

The project's key innovation is the positive detection of small molecules by a lateral flow immunoassay that is both highly sensitive and simple to use. Lateral flow tests using antibodies for the detection of specific molecules have been used successfully in other sectors such as biomedical and Agri-Biotech but this project makes the highly innovative step of using the format to design a rapid test that produces a positive signal in the presence of trace levels of explosives.

The SALIANT project comprised research and development of sampling and detection methods, technology integration and demonstration of the device prototype in forensic laboratories and first responder scenarios. A key feature of the project was the early introduction of an information feedback loop to guide the technology development. A process for gathering end user requirements throughout the course of the project and being able to factor this information back into the design of the device was implemented to good effect to minimise shortfalls in final prototype design. This has been of particular importance in, for example, focussing on the nature of the final devices with respect to multiplexing where, somewhat surprisingly, an optimised multiple stick device was favoured by end-users.

The overarching focus of the project was simplicity and sensitivity. The primary aim was to develop detection methods which are not only extremely simple to use and carry but are also fast and simple to make, thereby developing a platform technology which could be rapidly adapted and applied to new threats as they arise across a wide network of end users. In this SALIANT was most highly successful. Philip Ingram MBE, a senior British Military Intelligence Officer with extensive operational experience dealing with explosive and other threats and the intelligence exploitation of these threats, built on a CBRNE academic background, has worked closely with and reviewed the Selective Antibody SALIANT work and makes the following observations:

The Selective Antibodies small molecule lateral flow detection capability is one of the most exciting innovations in recent times. Its strengths lie in its ease of use, simplicity, sensitivity and accuracy.

The benefits provided by a first responder team being able to test for and report on the presence of explosive residue or other small molecules at an incident and report findings back to follow on specialist support teams is immense as it will allow specialise support to be prepared for the threats they may encounter thus allowing faster reaction and greater potential to save life and property. The forensic capability provided by being able to test for substances and confirm concentrations without sophisticated equipment, laboratories or training is unique and provides the ability for incidents to be exploited in a way where threats can be reduced.

The other potential uses for this technology mean it is not restricted to a narrow area of use but could provide simplified testing methods for a wide range of substances in a wide range of different scenarios. This combined with the relative low cost, long shelf life , no maintenance and virtually no training costs make it one of the more cost effective new technologies on the market

2.4 Project Objectives

The SALIANT project consisted of a number of clearly defined high-level objectives that together provided a framework for developing a series of work packages to develop the project.

The main objectives of SALIANT were:-

- Develop a protocol for the non-destructive sampling of target explosives, compatible with the lateral flow test strip format.
- Develop the proprietary universal immunoassay technology platform for the detection of explosive targets (RDX, HMX, PETN & TNT).
- Explore the capability for the simultaneous detection of multiple explosive targets using lateral flow strip and biosensor formats
- Develop a digital read-out display, storage method and means of communication of detected results generated by the test strip.
- Design a manufacturing process for the production of commercial quantities of test devices and produce test batches for both laboratory and field tests
- Evaluate the SALIANT technology in both laboratory settings and field trials by end users in Forensic science and First responders and provide feedback for further modifications and improvements
- Promote the research outputs and technological benefits through appropriate communication channels which are consistent with the guidelines for Security Research

- Organise workshops and training within the sector and develop strategic plans for post project commercialisation.

2.5 The SALIANT consortium

The SALIANT consortium consisted of 11 partners from commercial organisations, academic institutions and government forensic laboratories. Each partner was selected on the basis of their ability to contribute their relevant technology skills, sector knowledge and commercial know-how to each work package for the development of a hand-held device for real-time analysis of trace levels of explosives.

The organisations and their contributions to the project are briefly described below:-

Selective Antibodies Limited (SAL) United Kingdom: SAL specialises in developing lateral flow test strips based on the 'lock and key' interaction of an antibody with its target. Tests which use this are known as immunoassays. Specifically, the company is focussed on the development of high performance rapid tests for analytes of low molecular weight, such as explosives. Classical immunoassays are challenged by such target analytes, showing their presence by a *reduction* in observable signal. To overcome this SAL has invented and developed novel positive-read-out technology (showing an increase in observable signal with increased target analyte). This technology forms the core of SALIANT.

OY REAGENA Ltd (REAG) Finland: Reagena provided the technology and knowledge to read the signals from the SAL test strips. They provided a mobile hand held lateral flow test reader that streamlines the whole process of testing, validating, documenting, and recovery of the test results

Indicia Biotechnology (IND) France: Indicia's input was aimed at facilitating transference of the SAL technology into final -stage manufacturing of LFD tests. Indicia Biotechnology, brings expertise in assay development and OEM reagent manufacturing, the design and the manufacture of protein-activated microspheres and particle-based diagnostics and multiplexed assay development

Applikon Analyzers (APP) Netherlands: APP specialises in wet chemical analysis with a focus on the development of wet chemical analyzers, sampling and sample conditioning techniques. Applikon Analyers developed the air-to-liquid sampling technology.

Stichting Dienst Landbouwkundig Onderzoek (DLO-FBR) Netherlands: DLO-FBR has expertise in small molecule detection and supported the multiplex detection strategy by developing a microfluidic interferometry biosensor sourced from the Dutch company Ostendum

Forensic Science Laboratory (FSL) Department of Justice and Equality, Ireland : FSL provides a full scientific service from crime scene to court to various agencies and contributed to the specification process for the detection system and to the field trial methodology and evaluation process.

Netherlands Forensic Institute (NFI) Netherlands: NFI uses state-of-the-art technology and science to provide high-quality forensic services to clients within the criminal justice chain, such as the Public Prosecution Service and the police. Their contribution to the specification process for the detection system and to the field trial methodology and evaluation process complemented those provided by FSL.

Zilinska Univerzita v Ziline (UNIZA) Slovakia: UNIZA provided research and planning support to investigate all relevant issues that may affect future up-take of SALIANT technology. They designed and implemented field tests through collaborations with Fire and Rescue Service First Responders in Zilina.

Centre of Excellence for Life Sciences Ltd (CELS) United Kingdom : CELS is a not for profit organisation providing specialist support to universities and start up companies in technology commercialisation. They were responsible for spreading awareness of the project results and developing a plan for future commercialisation.

Newcastle University and KITE Innovation (Europe) Limited, United Kingdom were jointly responsible for the overall project management activities.

3. Science and Technology Results

3.1 The Aim of SALIANT

The aim of the SALIANT project was to develop simple, reliable, portable, easy to use tests for mobile and real-time detection of the explosives, RDX, HMX, PETN, and TNT to allow end-users in Forensic Laboratory services and First Responders at major crime scenes to rapidly, safely and easily collect evidence to inform immediate and follow up actions.

The focus for test development was ease of use and simple to make, thereby developing a platform technology within Saliant which can be rapidly applied to the detection of new threats as they arise from substances such as other explosives, toxins, drugs, pesticides and pose risks in areas including human and animal health, food security and environmental contamination.

This aim has been achieved by development of three essential elements; a sampling methodology, a detection technology and a reader to display and store results. Key to, development of the explosive detection system was input and feedback from the Forensic Laboratory services, end users and first responders.

The picture below shows the major outputs of SALIANT, a defined sampling procedure suitable for the post blast emergency situation and usable by 1st responders in protective equipment with a simple, rapid and clear test showing a single control line, to indicate that the test has functioned correctly, but that no target is present and a second positive test line in the presence of target. Finally a hand held reader to display and store the test result.

Figure 1. The Positive Detection System for Explosives Developed in SALIANT

3.2 Partners' Expertise and Contribution

The SALIANT consortium included 5 main science and technology partners from 4 EU countries with input from an end user group consisting of 2 forensic science laboratories from 2 other EU countries along with a team of first responders .
The Technology brought to SALIANT by each science and technology partner and how this addressed the SALIANT objectives is outlined below.

Selective Antibodies Limited (SAL). UK

SAL provided the core technology for detection of the target explosives. The lateral flow immunodiagnostic test (LFD) offers the user simplicity and the reliable real time results required, an example being the pregnancy test. There have, however, been very real challenges to bringing the full power of such technology to bear in the detection of small molecules such as explosives, drugs and the like.

There have been two challenges to overcome, first, generating antibodies to small molecules is difficult and requires specialist knowledge and practical skills, and secondly overcoming the problems of the normal classical 'competiitve-format' immunoassay systems for small molecules in which the presence of the target is measured by negative-readout by a *loss* in signal, so the more target present the lower the signal. Thus at zero analyte concentration the system provides its highest signal and at low analyte concentration only a little less than that. This makes the test less reliable and less sensitive as often the reduced signal from a low level of target cannot be distinguished from an almost equally high zero background measurement. The key innovation is the positive detection lateral-flow test for small molecules proprietary to Selective Antibodies Ltd in which a positive signal is generated as analyte concentration increases and ideally there is no observable signal in the absence of the small molecule target and a clear observable signal in the presence of the target. This is achieved by detection of the antibody only when the binding site is occupied by target analyte (a description of the core technology with an animated video is shown at <http://selectiveantibodies.com/index.php/the-specialist-technology>.

Based on this approach Selective Antibodies Ltd had defined two positive detection systems for small molecules, the Universal and Apposition systems. The Universal system has simpler components and is best suited to development of lateral flow test strips that detect a single target, whereas the Apposition system is more complex but provides additional potential to detect the presence of one of a number of target molecules, producing 'dualplexed' or 'multiplexed' tests which will allow the user to test for the presence of a number of small molecules on a single strip.

Lateral flow (or dipstick) tests developed in the first phase of SALIANT utilised the Universal system and were designed to detect a single target molecule. Later development investigated the use of the Apposition system along with the Universal systems to develop tests where the presence of a number of target molecules could be detected at once.

Stichting Dienst Landbouwkundig Onderzoek (DLO-FBR.) NL

Participant DLO-FBR investigated the performance of the microfluidic interferometer biosensor with the antibodies and positive detection systems provided by SAL. The advantage of the biosensor technology is the continuing potential for the development of extremely sensitive assays for small chemical explosives and drugs taking advantage of the same reagents used in the simple lateral-flow dipsticks which form the core of this proposal.

The essential innovation in this technique is the combination of an integrated optical interferometric sensor with antibody-target interaction. The sensitivity of the lab-on-a-chip sensor, that can be read out in a portable detector, approaches

detection of a single virus particle being, in principle, approximately 100 times better than other sensor techniques. The nature of the sensor enables multiplexed detection of many target compounds at the same time.

DLO-FRB led on the specification process for the SALIANT detection system leading with Applikon investigations into sampling procedures.

Applikon Analysers (APP). NL

Applikon Analyers developed the air-to-liquid sampling technology. APP had developed the MARGA; a monitor for measurement of gasses and aerosols in ambient air. One very important part of this analyser is the sampling of air and dissolving the constituents into solution in order to do the analysis. Development of this sampling technique is necessary in order to be able to apply it in other applications than ambient air.

OY Reagena Limited (REAG). FIN

Reagena provided a mobile hand held lateral flow test reader that streamlines the whole process of testing, validating, documenting, and recovery of the test results. Reagena has expertise in integration with secure communication networks to develop refinements in the connectivity solutions to adapt to the user needs and allow operators to perform the tests virtually anywhere.

Indicia Biotechnology (IND). FRA

Indicia input was focussed on the development of lateral flow tests to scalable manufacture. To deliver a test system design of the test must be integrated with the design and development of the reader. The SALIANT project constitutes an opportunity to advance the state of the art in LFD development. It also provides access to technology that will increase the range of small molecule targets in the point-of-need market.

End User Groups

Forensic Science Laboratory (FSL) ROI.

FSL contributed to the specification process for the detection system and to the field trial methodology and evaluation process. They conducted very important laboratory testing of the lateral flow tests. Forensic examination and analysis is carried out for all key physical, chemical and biological evidence types. The Laboratory is a founding member of ENFSI (European Network of Forensic Science Institutes) and active in the working groups for DNA, Drugs, Explosives, Fibres, Firearms, Fire and Explosion Investigation, Marks, Paint and Scene of Crime.

Netherlands Forensic Institute (NFI) NL

NFI contributed to the specification process for the detection system and to the field trial methodology and evaluation process. NFI also conducted critical laboratory testing of the lateral flow tests and oversaw the design of the field trials with end users followed by results evaluation.

University of Zilina (UNIZA) Slovakia

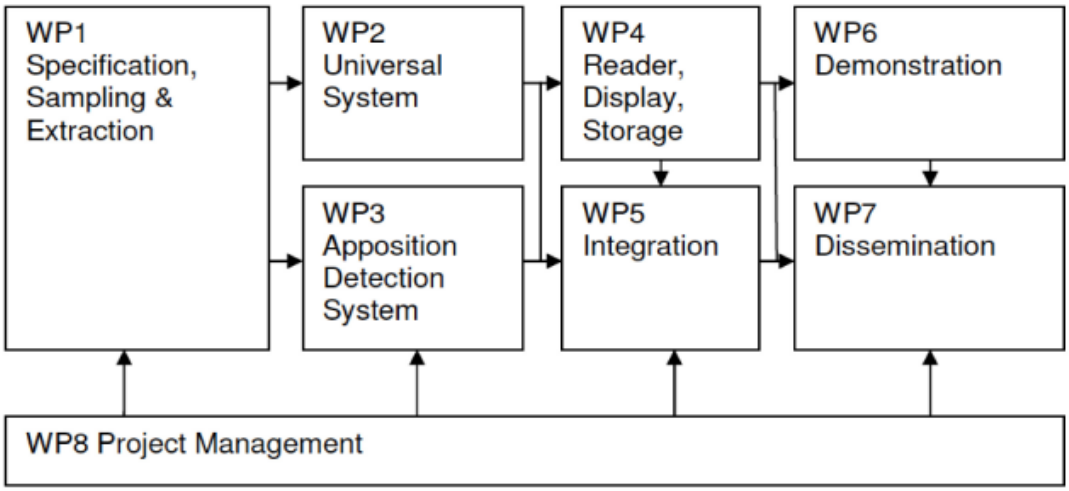
UNIZA brought expertise in crisis management strategies, evaluation of forensic technologies and first responder tests through Regional Fire & Rescue Service in Zilina. UNIZA advised on the design of the sampling methodology and lateral flow test required by end users and arranged for First Responder filed trials of the lateral flow tests to take place through the Regional Fire &Rescue Service in Zilina.

3.3 Integration of the Science and Technology Work Packages

The projects objectives were closely defined under specific work packages. The schematic below shows the 5 Science and Technology (S&T) Work Packages (WPs), including the technology content of each and the intended project workflow.

This also broadly indicates the chronology of the Saliant project. At the initiation of Saliant work began with WPs 1, 2 and 4 which encompassed the 3 essential elements of the project, sampling, detection system and reader. Once data and materials were available from the initial WPs, WP3, WP5 and WP6 were initiated.

Figure2. The SALIANT Work Packages (WP) and Project Workflow



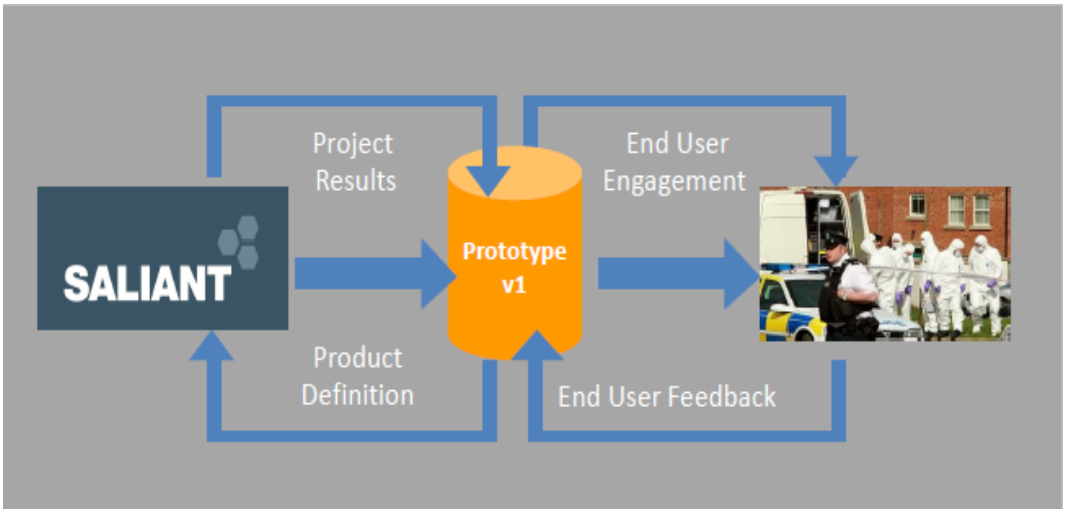
The lead consortium member for each Work Package and key partner interactions and contributions were:-

WP1: Led by DLO-FBR; NFI, UNIZA, FSL and APP were involved in the specification of the detection system for end user needs including sampling and development of portable air-to-liquid sampling system.
WP2: Led by SAL with input from NFI and FSL on the requirements of lateral flow tests for field use. Development for manufacture was conducted with IND.
WP3: Led by SAL for development of the apposition system. The options for multiplex detection were investigated for lateral flow by SAL and for the biosensor by DLO-FBR.
WP4: led by REAG for development of the reader with display, storage and communication capabilities.

WP5: led by IND; REAG and UNIZA contributed to the design and production of optimised and ready-to-use lateral flow devices.
WP6: Led by NFI; FSL and UNIZA were involved in the demonstration activity and end-user trials of the SALIANT detection system.

Interactions between the individual WPs was encouraged to ensure the integration of the different technologies involved. This increased as the project developed forming an informal information feedback loop. Most importantly, the advice from end users was critical for the design and development of all the technologies comprising the final test system, as well as for the commercial development of the Saliant test system. The feedback loops are shown below. Whilst this resulted in a degree of reiteration the outcomes significantly benefited.

Figure 3. Project and Prototype Development Feedback Loops



The objectives of SALIANT were met by the results and the outputs described under each of the science and technology work packages. Important interactions between partners or work packages where the transfer of information, advice, results or components generated in one work package are necessary to start or progress another work package are explained below.

Work Package 1 (WP1)

The WP1 Objective: A prototype device and method to deliver target samples at a range and composition that is compatible with immunoassays.

The first objective of **WP1** was to specify the requirements for sampling explosives with an emphasis on non-invasive/non-destructive conditions and develop/compare sampling technologies for target explosives in the post-blast situation. As part of this, APP, NFI and DLO-FBR worked together to define the range for each target in post blast air and to identify the extraction liquids suitable for the explosives and compatible with immunoassay. Traditionally, post-blast explosive detection is focused on surfaces in the proximity of the explosion (Figure 4) and air sampling is not applied to determine the identity of the explosives used. Post blast

explosive concentrations in air were therefore not known. This was investigated and a report completed where the main conclusion was that traces of the explosives would be deposited on the various surfaces in the proximity of the explosion and that air borne explosive residues (vapour or particulate) released directly after the explosion would be dispersed very shortly after the explosion resulting in variable and very low levels in the air. For this reason, work started on development of both air and wipe sampling methods.
To analyse the efficiency of sampling methods during development the standard method (HPLC-UV) for analysis of explosives in samples from NFI was implemented at DLO-FBR.

Figure 4. Wipe Sampling by First Responders

For **wipe sampling** UNIZA reviewed and reported on available sampling technologies, methods and devices used in the Slovak Republic for detection of dangerous substances, explosives and drugs with utilization in Fire and Rescue Services. These devices were inconvenient for rescue teams wearing protective equipment (see Figure 4) and were generally slow and unreliable. Consequently DLO-FBR initiated development of a suitable sample wipe system as well as ongoing review of sampling devices for innovative solutions. As there was no published or established method for explosive sample collection from surfaces expert input was sought from NFI who provided several possible methods.

The next phase in development of the surface sampling method was to specify the solvent required to recover all of the explosive present while ensuring that this did not interfere with the detection of the explosive in the LFD. The choice of solvents and assay buffers depends on the nature of the specific explosive and on the characteristics of the specific antibody used in the immunoassay. In all cases this had to be determined experimentally in WP2.

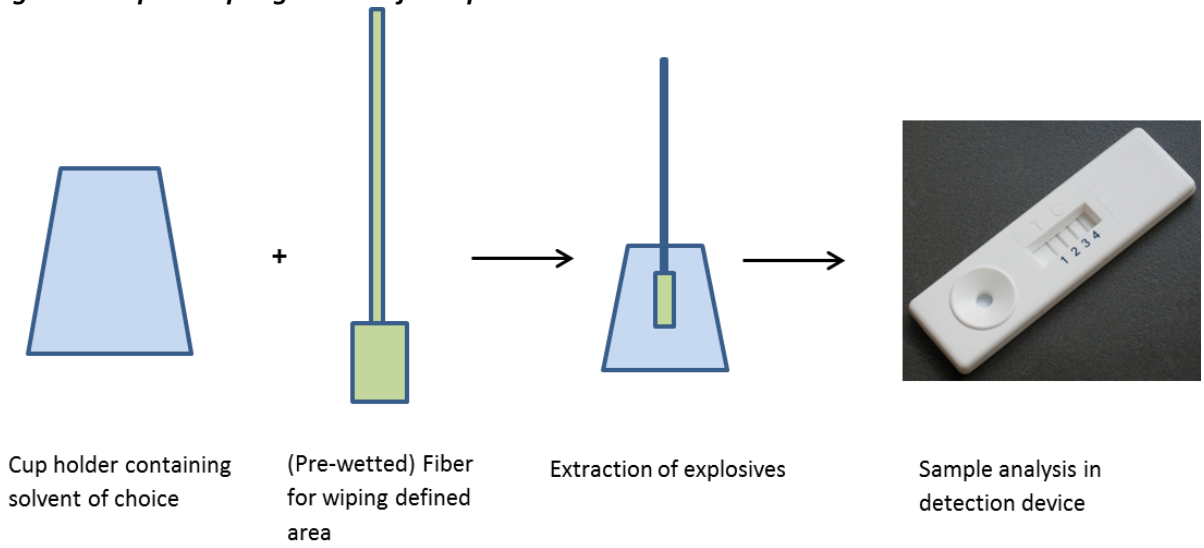
The choice of extraction buffers was restricted to (a) the solubility of the explosives in water and organic solvents and (b) the compatibility of these solvents with the antibodies/antigens used in the detection immunoassays. However three of the four explosives dissolve to some extend into water and the immunoassays can withstand a certain percentage of organic solvent. However this had to be determined for each explosive detection system.

Based on this information the following parameters were tested for their use and suitability in post blast surface wiping of explosive:-

1. **Type of wiping fibre**: traditionally used filter papers, gauze pads and polyester swabs known for their use in cleaning validation in industrial processes.
2. **Organic solvents for extraction from fibres:** i.e. methanol, ethanol, and 2-propanol. Use of solvent-water mixtures, which can be used immediately in immunoassays.
3. **Size of swabbing area** for a significant sample.
4. **Extraction time and method**
5. **Pre-concentration of sample** (possibly necessary for use in immunoassay)

Potential sampling methods were checked for suitability with the end user group in WP6 for use in the post blast situation. Input was used to refine the sampling methodology. High and reproducible recovery of the explosives was obtained using swabs and 30% ethanol. The sampling methodology was established and is shown in Figure 5.

Figure 5. Wipe Sampling Method for Explosives



This sampling method was applied in the field trials conducted by end users in WP6 and the recovery of each explosive was confirmed in the qualification study conducted in WP5.

For **air sampling** Applikon undertook re-design of an existing air-sampling unit to adapt the sampler to a simpler and compact design, which was an important aspect since the unit has to be easily transported and handled at the site of interest. Also continuous air sampling and a large air contact area was necessary to concentrate the sample in order to obtain enough of the explosive to dissolve in a low volume sample compatible with immunoassay. This device had potential to clean the sample and could cope with a range of sample volume, composition and temperature.

Extensive evaluation studies were conducted to define the design specification for the sampling device to extract explosives from both aerosols and gasses. While the

capture of explosives from gasses was efficient, capture from aerosols was found to be less than expected. Various filters to trap aerosols were tested and a range of solvents but it was concluded that re-design of the air sample was necessary.

The first modification tested was inclusion of a deliberate 'turbulent' design, the next was to provide aerosols with a hydro-coating with steam before they were absorbed and thirdly inclusion of a 'cyclone'. While technically challenging in a simple sampling device, this was deemed to be feasible and robust as there are no moving parts and the steam injector is proven in-house technology.

The air sampler comprised of three main parts with specific functions and the effect of modifications to each of these was tested against recovery from gasses and aerosols. The main conclusions for improving explosive recovery are below:-

1. The steam chamber. The length of this component has a small but significant effect
2. Inclusion of a cyclone has a small but significant effect.
3. Capture chamber (denuder) design. A column denuder filled with stacked 4mm glass spheres produced the highest recovery
4. Air Flow Rate over the range of interest did not affect recovery

A prototype air sample was built and tested. The function of the 'critical orifice' was tested at different temperatures, airflows and humidity and shown to be robust. The prototype air sampler gave an excellent 100% recovery with a control aerosol sample.

Work Package 2 (WP2)

The WP2 objective: Develop the Selective Antibody (SAL) Universal technology platform for the detection of explosive targets to the stage where this can be transferred to manufacture of prototypes in WP5.

WP2 was the core of the SALIANT project. This WP provided materials and information essential to initiate WP3, WP4, and WP5. At the outset of WP1 model lateral flow tests for targets other than explosives were supplied to REAG for development work in WP4 and model test components supplied to IND for initiation of WP5

Initial work included the design, development and sourcing of the three critical components of the explosive detection tests, the target specific monoclonal antibodies (MAbs), the target specific blockers and the capture antibodies (Abs).

As the panels of all 3 components for each explosive test were prepared the next stage was to test these in various combinations to demonstrate that it was possible to detect each explosive using the SAL Universal technology. This was the Proof of Concept (PoC) stage.

The first 2 of these components are common also to the SAL Apposition system. These were transferred to WP3 once available.

The preparation of the 3 critical components of the detection test for each explosive was conducted in parallel.

Isolation of Target specific Monoclonal Antibodies (MAbs)

Several specialist stages were required to achieve this objective.

1. Chemical modification of the explosives to generate and monitor the immune response
2. Creation of cell lines that produce a MAb
3. Screening of the MAb produced by the cell lines for the characteristics required for development of the detection test. These include how well the MAb binds to the explosive and how specific it is. This stage is critical.
4. Production & purification of the MAb, storage, viability and security of MAb cell line.

Suitable target specific MAbs were sourced (generated and licenced) for all 4 explosives, 4 for TNT, 4 for RDX/HMX and 1 for PETN.

Preparation of Target specific Blockers

Panels of target specific blockers were prepared by chemically coupling various several large molecules such as proteins and polymers to the target explosives followed by measurement of the effectiveness of the blocker. Some of these targets required a lengthy multistep synthesis of a linker between the explosive and large molecule to enable chemical coupling.

Sourcing Capture Antibodies (Abs)

The universal system is reliant on capture antibodies that:

1. Recognise and bind the target specific MAb.
2. Recognise and bind sites on the target specific MAb that can, in the absence of analyte target, be blocked by the target specific blocker.
3. Bind the target specific MAb proportionately in the presence of target and target specific blocker.

The challenge was to obtain a panel of Antibodies (Abs) that meet these requirements.
Testing of capture Abs was conducted in the lateral flow format incorporating both the target specific antibodies and target specific blockers.

Two routes were applied to source capture Abs, these were:

Immunisation of Goats to generate Polyclonal Antibodies (Pabs)

The advantage of this route was that Pabs recognise multiple sites, the disadvantage is that Pabs can be more difficult to block in the system. A successful immunisation will give a good supply of antibodies but this will ultimately need to be renewed and a degree of batch variability will occur. However, given the very small amount of such antibodies used on individual strips this is not usually seen as a major resource problem. SAL has isolated 2 sources for polyclonal antibodies, one commercially available and another internally generated from goat immunisation.

Both polyclonal antibodies have been shown to be effective capture Abs for all target specific MAbs.

Anti-murine antibody monoclonal antibodies (MAbs)

These antibodies have been generated against the target specific MAbs by applying the methodology for isolation of the target specific MAbs. They are easily blocked but are generally specific to the target Mab and, being so, usually require a different capture antibody for each explosive test. MAbs are a more secure option as they are produced in cell lines providing a continuous consistent supply. SAL developed a range of anti-murine antibody MAbs within the SALIANT project and combined those with a panel consisting of commercially sourced and previously generated in house and MAbs. These were screened against all target specific MAbs for the 4 explosives and several suitable candidates were identified. Once the 3 components were available the focus then moved to the next objective:

Proof of Concept (POC)

In this stage many combinations of the of the 3 components, the target specific monoclonal antibodies (MAbs), the target specific blockers and the capture antibodies (Abs) were combined into a lateral flow device (LFD) 'dipstick', and tested for the detection of the relevant explosive against the key requirements of the detection test.

The key requirements for a successful test were a clear positive signal in the presence of explosive and a clear negative in the absence of explosive and detection of only the target explosive. PoC was demonstrated for all explosives. An example is shown for TNT in Figure 6. This shows a clear negative (blank) and clear positive with TNT (test), the other explosives, PETN, RDX and HMX reading as negative. A major added bonus of the technology was seen at this stage in terms of the surprising speed at which the first signs of a positive signal were observable on the devices with Figure 6a showing clear detection of RDX in one minute. As these are seen in terms of a red line coming out of a blank background these are much fast and easier to interpret than with competitive-format systems.

Figure 6. Lateral Flow Devices (LFD) to Detect TNT

Negative Positive **Specificity for TNT against other explosives**

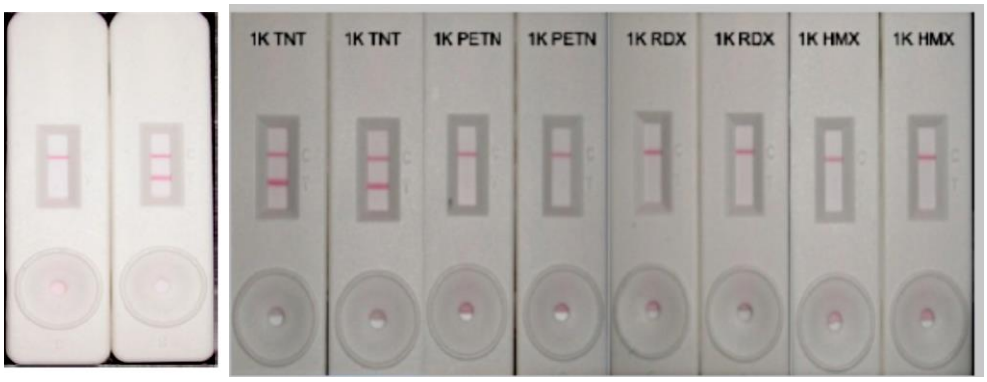


Figure 6a Ultra-fast detetction of RDX showing positive detection in one miniute

Optimisation

Optimisation was the stage where the best combination and amounts of both the biological test components, the target specific MAbs, target specific blockers and capture Abs and the hardware, such as sample pads and the like that makes up the LFD are determined and fixed for transfer to manufacture.

During this stage information is gathered on performance such as the amount of explosive detected and whether there are interfering substances that either cause a false positive signal or prevent detection of the target explosive.

Input from WP6 end users was critical to highlight the nature of samples in the field and any likely interfering substances and input from WP5 was important to ensure that the optimised detection tests were compatible with scalable manufacture.

The optimised LFD for detection of TNT is shown as an example in Figure 7a TNT was applied across a range of amounts and this was detected proportionally to the level of signal clearly observable by eye (Figure 7a) and, as shown by a different series of experiments, by means of the Reagna reader (Figure 7b).

Figure7a. The Optimised TNT LFD showing increase in signal with increased TNT

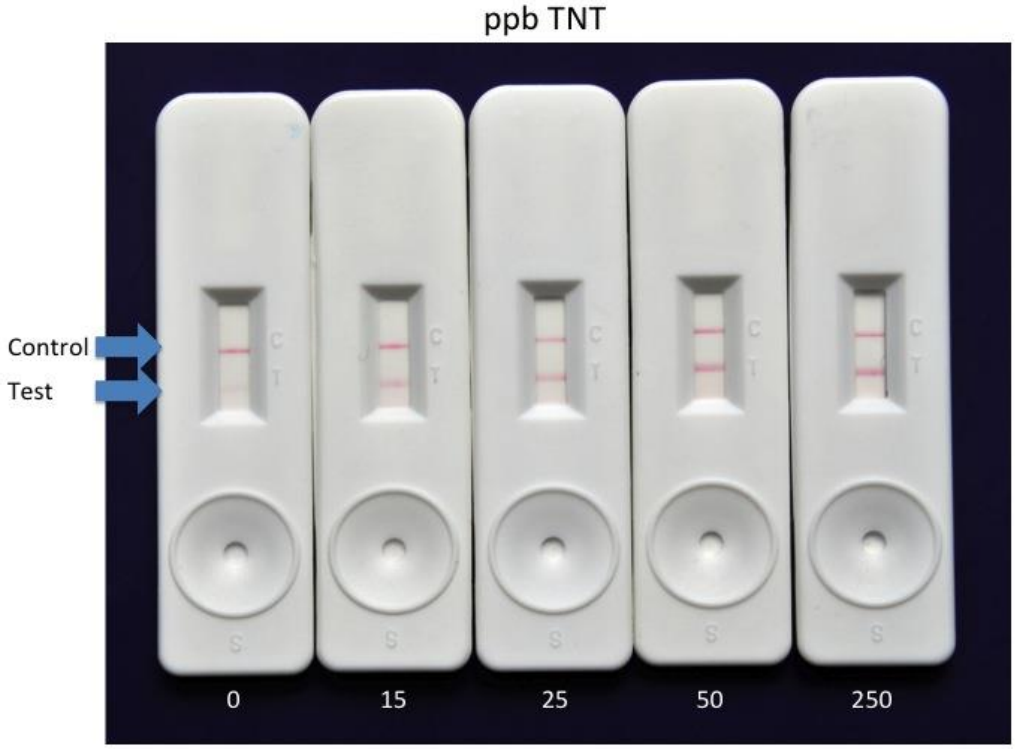
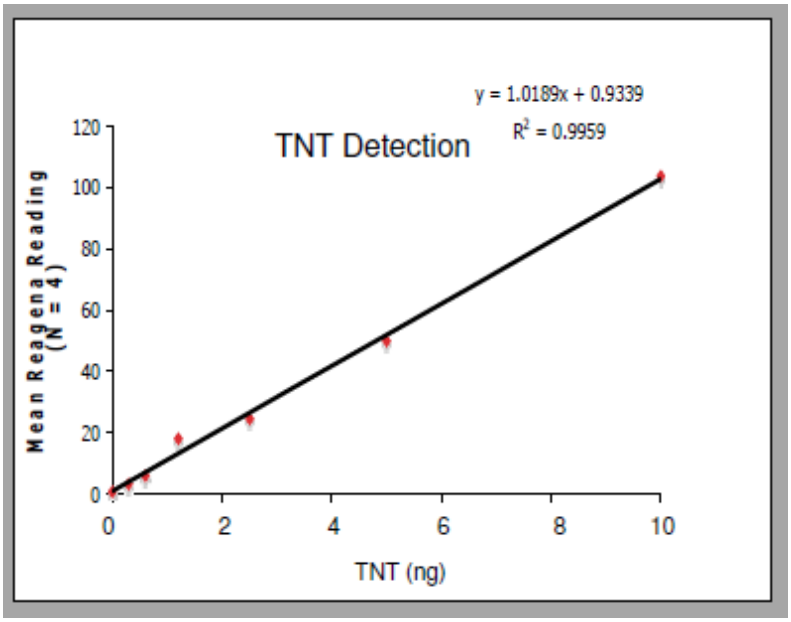


Figure7b. Quantification of TNT Lateral Flow Devices for TNT



A major challenge during optimisation was modifications to the hardware components of the LFD necessary for samples containing environmental contaminants such as asphalt, rust and soot and a range of powders such as flour and detergents. These samples were representative of those likely to be sampled in a post blast situation. Asphalt, rust and soot caused a positive result in the absence of target explosive due to the presence of particulates in these samples. The hardware components of the LFD were optimised resulting in clear negative

results in the absence of target explosive. Similarly, success was also achieved with the other explosives of the programme: RDX, HMX and PETN.

Transfer to Manufacture

In demonstrating that the methods to produce the LFDs were ready for transfer to manufacture in WP5, 3 batches of each LFD were prepared and tested. These were shown to be consistent and enabled the production process methods to be transferred from SAL to IND. To ensure full understanding the lead technical person from IND was invited to observe the production processes at the SAL facility. This work was continued in WP5.

Dual/multiplexing

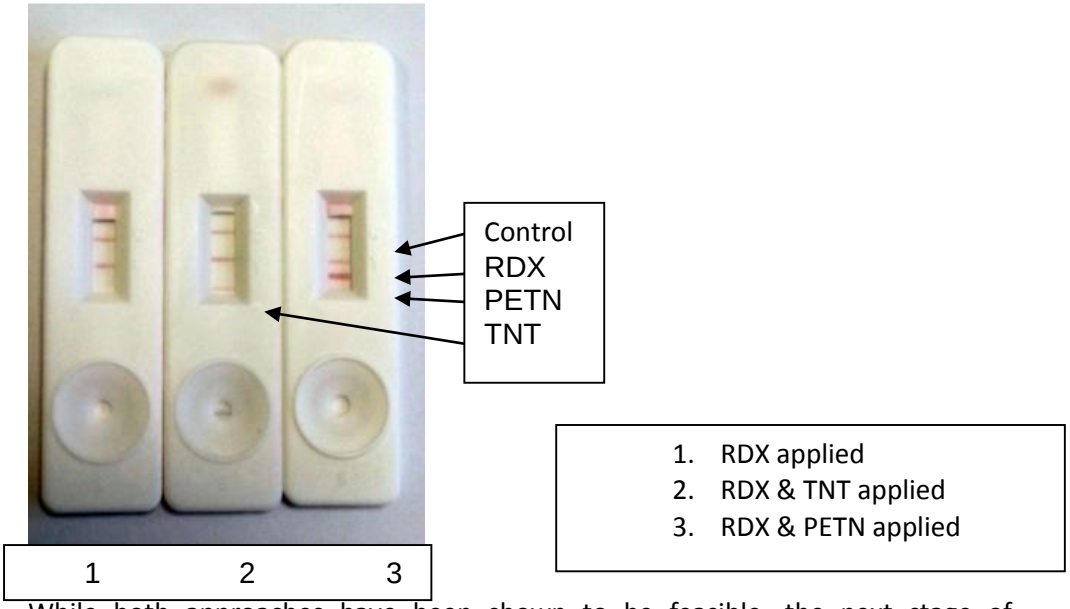
At the outset of SALIANT it was believed that the best route to detect more that 1 target would be by applying the Apposition detection system in WP3. However during development of the Universal detection system it was found that dualplexing, (detection of 2 explosives) , or even multiplexing, (detection of more than 2 explosives) may be possible with the simpler Universal system.

There were two ways in which more than 1 target could be detected.

1. A test that would detect all 4 explosives with the single test line appearing when any of the explosives was present. Such a test would alert 1st responders to 'Danger one or more of the 4 target explosives (TNT/PETN/RDX/HMX) present',

2. A test that would detect and identify the individual explosives present such as by the position of individual separate test lines as in Figure 8

Figure 8 . Multiplex Detection of Explosives



While both approaches have been shown to be feasible, the next stage of optimisation would require extensive studies as optimisation would be required for the combination of targets detected with different sample combinations. Our

studies to date have also highlighted the problem with dividing the maximal (limited) amount of gold that can be applied to a stick between a number of different analyte systems on the stick – by definition, each one receiving a fraction of that it would have received if the stick were for a single target analyte. This consideration, plus the clear fact that no one system on a multiple stick will be optimised only for itself led us to start favouring a multiplexing system in which individual sticks for each explosive are optimised and placed together in a single cassetted array for the end user. This is a standard industry format. A single sample can be applied across all tests and the presence of each target determined.

The views of end users were sought on a preferred format for a multiplex test, in general the latter option of an arrayed test to detect all 4 explosives was preferred as the next development. This also has the advantage that the current reader would be suitable without modification (the reader and results display would require modification with the previous options).

Work Package 3 (WP3)

The WP3 objective: Develop the Selective Antibodies Apposition technology platform to
mitigate the risk of reliance on the Universal system (WP2) ; expand the detection capability to multi-target detection tests and broaden the detection capability to a range of existing and developing test formats

WP3 comprised of 2 technologies, firstly the SAL Apposition detection system and secondly, a research stage biosensor technology platform from DLO-FRB. Work was aimed at exploring the potential of the development of multiplexed detection tests where multiple targets could be detected in the same sample, for the 4 explosives.

Apposition (SAL)

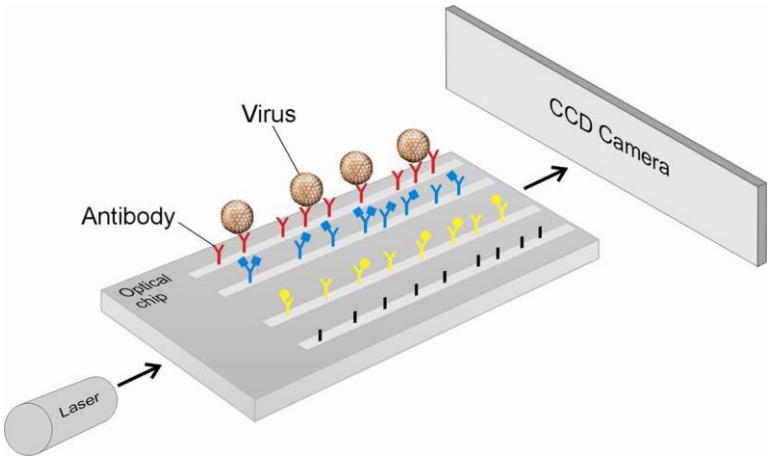
Development of an Apposition detection system requires 2 components common with the Universal detection system, the target specific monoclonal antibodies (MAbs) and the target specific blockers supplied from WP2. However the target specific MAb must be chemically modified to attach a detectable entity close to the explosive binding site on the MAb while retaining the ability of the explosive target to still bind to the MAb. This requires highly specialised proprietary chemical knowledge and practical expertise. This was achieved for all 4 target explosives. The chemically modified target specific MAbs are called Apposition reagents or conjugates. PoC was shown for detection of explosive targets in 2 immunoassay formats, lateral flow and ELISA.

Multiplex detection can be achieved by utilizing different detectable entities to produce the Apposition reagents for each target in combination with a capture antibody that binds only the detection entity for that particular explosive. However, as a result of the very real success in WP2 for both single and multiple target detection with the Universal detection system plus the feedback from end-users, there was no longer risk on relying on the Universal detection system to achieve the SALIANT objectives. Therefore, especially as the Universal detection system is simpler with respect to manufacture, effort was focused on this route.

Biosensor (DLO-FRB)

This technology has the potential to provide quantitative detection systems that are extremely sensitive, are rapid, and can detect many 10's of targets at the same time.

Figure 9 . Schematic of the Biosensor

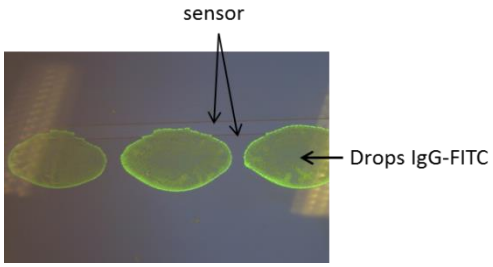


The detection system works by attaching antibody to a silicon surface. Many different antibodies can be attached in different positions/channels to allow multiplex detection. The silicon surface has a laser light beamed across its surface that is detected at the opposite end of the silicon chip by a CCD camera. Sample containing target/s is introduced into the chip and flows across the surface though the channels. When target (a virus is shown in the Figure 9) binds to the antibody an interference pattern is generated in the emitted light that is captured by the camera. The change in light pattern is proportional to the amount of target bound and the position can identify the target detected.

The system works well for detecting antibody binding to large molecules such as proteins, however, as explosives are small chemicals the change in signal would be too small for detection. This problem could be overcome by applying the SAL Apposition detection system to the biosensor format. The target explosive is detected by binding to the large apposition reagent that is then captured by an antibody attached to the surface that binds to the chemical label.

The R&D biosensor method had to be modified regarding assay time and reagents used as one assay took approx. 2.5 hours and required 1 ml of antibody solution. Continuation with this method would mean that enormous amounts of antibody would be needed for development and validation of the biosensor detection system. Therefore work started on printing the capture molecules to the sensor surface using a microarray printer, a non-contact dispensing system where ultra-low volumes of antibody solution were required. The distribution of capture antibodies on the sensor surface was uneven due to droplet formation. Figure 10 shows a doughnut-formation of the drops, the protein is concentrated at the edges of the drops.

Figure 10. Formation of Antibody on the Sensor Surface



A model capture antibody system was tested with the target in flow - over operation to determine the performance of the biosensor chip. Although the indication was that target detection was 100-fold better that existing commercial biosensors the results were not reproducible in that the binding of the target was different at each use after regeneration of the chip. Modifications were carried out to the chips but reproducibility remained unacceptable. The chips are expensive and next stage development of disposable chips is under consideration.

The biosensor was tested with the apposition detection system. Despite repeated attempts it was not possible to bind either the TNT or RDX specific antibody to the chip surface.
From these results it was concluded that further development of the biosensor chip is required.

Work Package 4 (WP4)
The WP4 objective: Develop mobile hand-held device systems for point-of-need/ point-of-site testing with software, which supports the universal and apposition test systems developed by SAL.

WP4 consisted mainly of R&D related to software engineering work. A model Universal system lateral flow strip was provided by SAL for development of the first prototype reader for the universal system.

This objective was successfully completed and a prototype portable reader with PC software capable of reading one test line and one control line suitable for single target explosive detection was supplied to SAL for test development in WP2 and WP3 and to FSL for laboratory testing in WP6.

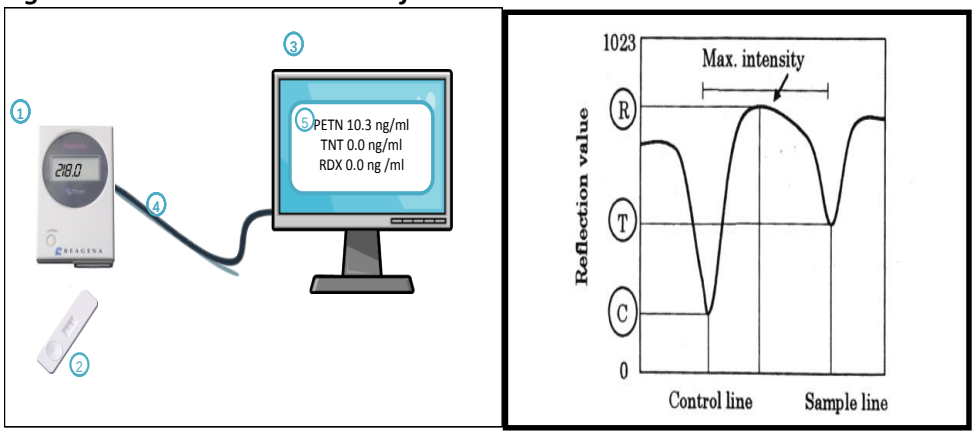
The next generation of reader was developed to include the capability to securely store and transfer results obtained in the field. The requirements and end-user preferences for these features were obtained from the results of an end-user questionnaire issued to a network of first responders as part of work package 6. The results were then discussed to determine the features to be developed in the second-generation model.

A draft workflow for reading measurements and storing data was produced (Figure 11). The system consists of a pocket-sized, battery equipped, optical reader (1) used for measuring the intensity of the test line appearing in a test cassette (2) where the intensity of each test line is proportional to the amount of explosive detected. The reader produces a series of readings, as numerical values that are transferred

to a personal computer (3) via a USB cable (4). These readings are processed by the software (5) to produce results indicating the amount of target explosive detected in a defined unit of measurement.

Modifications and additions were necessary in the electronic and mechanical components. A standard secure IEEE 802.11 wireless local area network was used for wireless transfer of measurement result data from a reader device to a portable PC workstation. Development included software for mobile phone or other portable device. Testing was performed using prototype cassettes pre-run with solutions containing RDX at various known concentrations.

Figure 11. The Reader and Workflow



The test cassette is inserted into the reader that scans this as it is ejected so that the control line is read first and the test/sample line next. The control line must be present to allow reading of the test/sample. This means that if the detection test has not run correctly for any reason then a test result is not given. This ensures the reliability of the test result be it positive or negative

Two prototype readers were produced for the field trials in WP6. These were calibrated against the scaled up batches of LFDs produced in WP5. Reagena participated in the field trials to monitor the performance of the readers and assist the end users the operation. Reagena also supported other WP's in relevant questions, such as WP5 in regulatory issues and WP7 in dissemination issues.

Further development of the reader continued to support the reading of up to 4 test lines in the same cassette for multiplex target detection. To guarantee the accomplishment of this task close interaction with SAL has been necessary to read cassettes with multiple lines and develop the software for interpreting them.

Work Package 5 (WP5)

The WP5 objective: Integrate the reader designed in WP4 with sampling and detection technologies determined in WP1 and define an optimum design for manufacturing ready-to-use lateral flow devices.

The design of the lateral flow test for manufacture ensured that the format of the test and the components (type of particles, membrane length, width and porosity, buffer compositions) were compatible with a scaleable manufacturing process,

suitable for use with the sampling procedure and devices and able to be read with the reader.

SAL provided IND with model test components so that they could become familiar with the process for producing a LFD based on the Universal detection system. Once PoC was demonstrated for the Universal detection system in WP2, SAL and IND worked together to ensure that the design requirements were foremost during the optimisation stage for the LFDs.

The efficiency of the transfer process from WP2 to WP5 was validated with a production of a TNT pilot batch of test strips. Minor modifications to the production process were identified to enable scalable manufacture, the most significant being the method of deposition of test components onto the strip, which had to be adapted to suit the injector equipment used for scale up. Materials for batch production of test strips were identified at this stage as were the optimal dimensions of the prototype device.

The performance of the TNT pilot batch was comparable with those produced by SAL. The pilot lots were sent to NFI and FSL for evaluation in laboratory test and proved to be equivalent to those developed in WP2. Pilot batches of all tests were produced and tested and shown to be equivalent. The manufacturing process was established and the methods documented.

Larger scale batches (1000-2000) of each test were manufactured to demonstrate the scalability of manufacture process and provide the tests used in the field trials in WP6.
A technical file was prepared for each test containing all the information required to manufacture, and use the prototype product.

The performance characteristics and stability of the prototype tests was determined in a Qualification Study. This defines any factors that are likely to affect the reliability of the test results and the operational limits of the test.

In summary, all tests are stable on storage between cooled and ambient temperature and can withstand elevated temperature for a period exceeding the maximum anticipated during extenuating circumstances, 1 month up to 45˚C. The tests can be run over the temperature range of expected climatic conditions 4 to 45˚C and for between 5 to 7 minutes pre-read.
The wipe sampling technique was confirmed to fully collect/recover TNT, PETN and RDX, but was less effective at 80% for HMX. Greater than 95% discrimination of positive and negative samples was obtained for all tests and this was unaffected by interfering substances (using the average of several tests).

Previously observed cross-reactions were confirmed: The RDX test strip detects HMX and the TNT test strip detects TNB. As both HMX and TNB are explosives this cross reactivity actually expands the range of explosives detected. The test limits were set for a blank and positive sample under laboratory conditions with a standard amount of explosive and these are used to conduct quality control checks on test batches. The results from field trials in WP6 indicated these limits under field conditions. The tests performed the same regardless of different operators or site of test.

In conclusion the detection tests for the explosives were considered ready for more extensive field-testing by first responders.

Work Package 6 (WP6)

The WP6 objective: Field Trial evaluation by end-users in Forensic Science in First Responder Scenarios to determine the effectiveness of the SALIANT system in scenario based evidence selection and information guided investigation.

End user survey

The input of end users from WP6 informed the work in both WP1 and WP4 from the outset of the SALIANT project. At the earliest opportunity a survey was made of first responders at post-blast scenes on their preferences for the design of a hand held detection device. A questionnaire was drafted and distributed through FINEX, the Explosives Working Group of ENFSI (European Network of Forensic Science Institutes). It was also decided that a framework should be prepared for testing a prototype hand held detection device once available.

The survey of "What do first responders want?" organised through the FINEX Explosives Working Group approached approximately 60 members including institutes in Europe and observers from outside Europe. From the responses several key requirements were identified. These were that the reader should be hand held with a lap-top interface, the detection system should cover a range of explosives. The testing device weight & portability, ease of calibration and assay time were all rated as highly desirable

Laboratory testing

A draft testing framework was prepared and agreed with the other partners. This covered laboratory testing of the detection systems during the optimisation stage in WP2 with samples which contained environmental contaminants and so were representative of those likely to be obtained in the post blast situation. This highlighted that contaminants including rust, soot and asphalt as well as a number of 'white powders' caused a background/positive signal in the test strips for all explosives. This was ultimately resolved with modification of the hardware components in the LFD as described in WP2.

A series of tests were conducted by FSL on the modified lateral flow test cassettes. These confirmed that positive results were obtained from the cassettes when expected i.e. when exposed to solutions of RDX, PETN and TNT and that false positive results were not obtained when the tests were exposed to commonly available "white powders" sometimes found in hoax explosive devices.

FSL advised that potentially ambiguous results may occur in the field i.e. faint red lines ('shadows') and numerical values on the reader which are higher than those obtained for 'clear negatives' but lower than those obtained from undoubted positives. This should be addressed by drawing up protocols which define clearly for the end user the criteria which must be met for a positive result, combined with appropriate training for end users. The following criteria were suggested:-

1. A clear red test line is required for a positive result; anything else is to be regarded as negative.
2. A numerical cut-off point for a positive result to be defined. Anything which gives a value below the cut-off point is defined as negative; anything above the cut-off point is defined as positive.

This advice was taken into account for test qualification in WP5 where limits were set for blank and positive samples for each explosive test system. Optimised detection systems were made available from WP2 for further laboratory testing which was conducted on artificially generated post-blast debris. The results confirmed the utility of the Saliant detection systems.

Field testing

When pilot batches of LFD had been manufactured in WP5 field-testing was conducted culminating in actual post-blast testing. This was conducted against a defined methodology for performing field tests in an area prepared for field trials according to selected scenarios.

Specialist pyrotechnists performed the explosive blasting. Three blast scenarios were staged and extensive sampling and testing for the presence of explosive over the blast area was carried out by end users from the Zilina Fire & Rescue Service. This was followed by comparative laboratory analysis of the samples by NFI.
The three scenarios were as follows:-

1. booby-trap explosive system at the open area,
2. booby-trap explosive system nearby building object,
3. booby-trap explosive system under motorcar.

Climatic conditions for the three scenarios varied from around zero degrees C to 22˚C.

The objectives of the field trial were to evaluate the practical aspects of:-

1. Taking samples with swabs
2. The explosive extraction procedure
3. Testing the lateral flow cassettes and reader use

Video and photo documentation was taken for all field tests.

Figure 12: Field Trial Sampling and Testing

The main observations from the field tests were that the lateral flow devices, the current sampling protocol and reader behave well and are usable under field conditions. High sensitivity and a low rate of misfunctioning approx 1% were reported. In real life scenarios with untrained personnel, it was advised that ideally the number of steps in the protocol should be reduced.

Conclusion

The RDX LFD performed very well in field conditions and provided a reliable indication of the presence of traces of RDX in real conditions. The PETN LFDs are slightly sensitive to interfering substances giving rise to a background reading for blank samples. Background reading was observed more strongly for the TNT LFD. To ensure reliable detection of these explosives the numerical value cut off for the blank can be increased, meaning that only tests reading above this would be reliably positive. This would mean that very low levels of the explosive may not be reported but as these levels are very much lower than the amount of explosive likely to be found in post blast scenarios this would have little practical consequence.

4 Dissemination Activities

4.1 Aims and objectives

The information dissemination objectives for the project were to communicate research results obtained from all parts of the project to a wide and varied target audience. SALIANT used a variety of communication tools to reach those audiences with a number of different key messages.

With the proviso of suitable security of dissemination, promotion of results has already created awareness and led to the establishment of connections with a network of contacts within the security sector for future exploitation and development of SALIANT technology and products.

4.2 Target audience or group

There were four main audience groups for whom this project would have an impact. These are described below with a brief explanation of the type of impact expected.-

1. **Security Industry:** The primary objective of the project is to produce a novel diagnostic device for the security industry with the potential to make a positive contribution to the way forensic investigations are carried out in the future. End users in this group form a distinct and highly niche target audience. Information dissemination to this audience has been specifically targeted with the help of the partners and advisors.

2. **Academic Community:** The principles of the new positive lateral flow technology and underlying science are applicable to the rapid detection of small molecules in other sectors such as healthcare and agricultural biotechnology. Building awareness in this community may stimulate discussions for future collaborations and new research in this area.

3. **R&D and Manufacturing Industry:** Lateral flow tests using biological reagents for post blast forensic investigation is a promising product category. Future commercialisation and market uptake of high performance devices would be expected to have a positive impact on the companies manufacturing these devices including their respective supply chains - offering new revenue opportunities for this market sector.

4. **General Public**: Bringing the project and its aims to the attention of the general public throughout the EU and globally using various communication channels

4.3 Dissemination Filter

The security nature of the SALIANT project necessitated that both dissemination and exploitation of project results adhered to the EU Template for Security Research. Outright publication has therefore been restricted. All project outputs adhered to this criteria and an additional internal classification was applied according to content and disseminated only to appropriate audiences.

The dissemination classification was divided into four categories:-

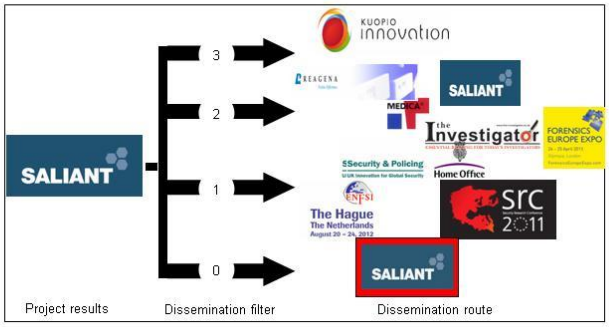
0- Highly sensitive information for **EU and consortia members only**

1- Sensitive information concerning methods and results for the **security industry only** disseminated to invite discussions and generate feedback

2- Scientific and technical information which may have broader application and may be useful to the **academic community and supply chain manufacturers**

3- Non technical information for the **general public** and the media

Figure 13 : shows how the security filter was implemented to direct dissemination at the various events where SALIANT was promoted.



4.4 Marketing Materials

The SALIANT project developed a number of marketing materials which it used throughout the course of the project and some of which will continue to be used as the technological capability and programme develops further. These are briefly described below.

SALIANT Trademark Report

Figure 14: The SALIANT logo

"SALIANT" is an acronym describing the project's underlying technology and as such would be regarded as an invented word and deemed registratable as a trademark. An initial trademark search carried out by UK patent agents found no existing registration of "SALIANT" clearing the way for a future registration. Trademarks including logos (Figure 14) once examined and approved are registered indefinitely with renewals every ten years. All 27 countries within the EU can be covered by a single trade-mark application Registrations are for specific use in certain classes of goods and services. e.g. :- Class 9: apparatus for the detection, sampling, diagnosing and analysis of explosives; read-out apparatus for detectors; etc.

SALIANT website (www.SALIANT.eu)

The website URL was registered at the beginning of the project and will remain live at least until 2015. The site has a member's area for posting internal project documents which but was used primarily as an external facing site to promote the project to the general public.

Initial setup of the site did not include any search engine optimisation techniques to elevate its position in search rankings. This was later reviewed by the consortia and the site now contains a list of relevant search words and phrases. This has had the effect of raising the site's prominence in searches. Website analytics for 2013 recorded just under **20,000 hits** and around **1,000 unique visits** with significant interest from non-EU countries.

SALIANT posters

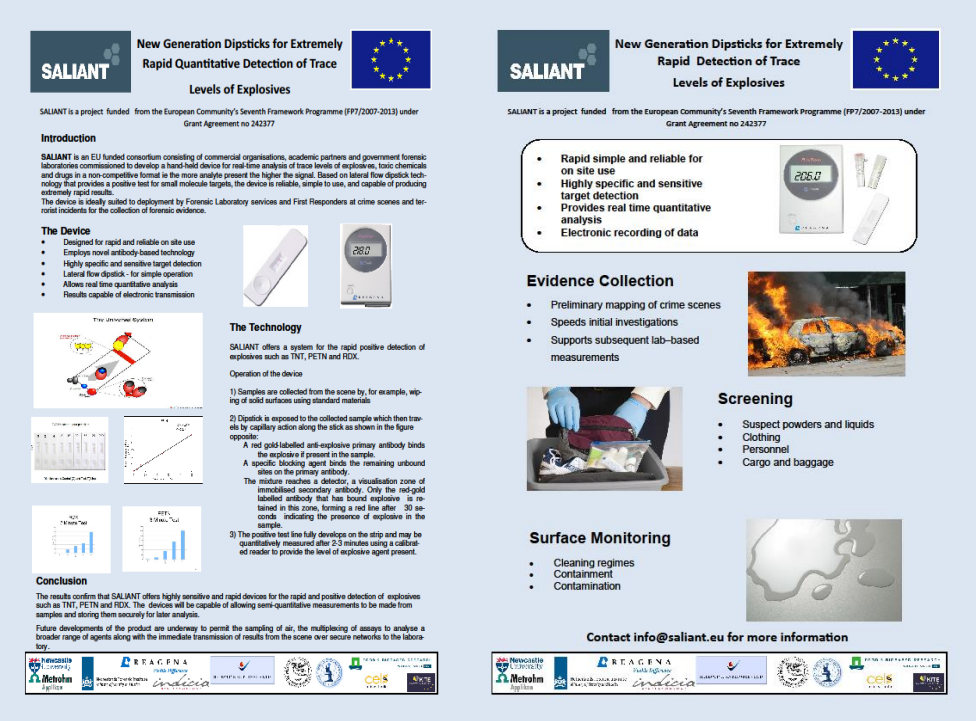


Figure 15: SALIANT posters

Two posters (Figure 15) were prepared in both banner and electronic format for general presentation purposes. A technology poster describes the SALIANT system and results of analysis of the explosives TNT, PETN and RDX. An applications poster describing potential uses for the system. Both posters have been used to good effect at various events in promoting the project and generating commercial interest.

SALIANT Publications

Academic

The following academic publications have arisen from the SALIANT project

	Colin H. Self, Stephen Thompson, Theresa Street, Kelly J. Lamb, Gordon Duffin, John L. Dessi (2013), Maggie Turnbull, **Non-competitive Immunoassays for Small Molecules - the Anti-complex and Selective Antibody Systems** ,Chapter 2.2, p. 61, Elsevier, Ed. David Wild. (restricted publication for security reasons)
	Anton Osvlad, Jozef Svetlik, Jaroslav Flachbart, Maria Luskova, **Project SALIANT - Field Tests,** EDIS, University of Zilina, in press

Trade Press

Feature

SALIANT SHOWCASES INNOVATIVE HAND HELD DETECTION DEVICE

An innovative hand held device that can detect small traces of explosives at a crime scene or terrorist incident is being showcased for the first time in public at the Forensics Europe Expo conference.

The SALIANT Project has been developed by a European consortium of biotechnology companies, universities and government forensic laboratories and uses novel immunoassay technology to transform the collection of vital evidence in 'real time.' The project brings together expertise from the UK, Finland, France, Ireland, Slovakia and the Netherlands and is funded by the EU Seventh Framework Programme (FP7) which aims to fund pre-competitive research within the European Union.

Scientists have spent the past three years developing the prototype mobile hand-held system through a process of laboratory development, end user engagement and prototype testing in both controlled and field environments. The current device has been successfully tested using trace samples of TNT, RDX and PETN.

The device is simple to use and is robust enough for use in the field. It requires minimal training and can be used by personnel wearing protective equipment. Samples can be analysed within three minutes reporting the presence of trace explosive material by digital display readout. The results are stored in the device for secure transfer at a later stage. One of its most innovative features is its highly novel yet simple positive detection lateral-flow test for small molecules that is highly sensitive and easy to use by both investigators and scientists. This is considered a real breakthrough as in the past, a positive readout signalling the presence of small molecules has not been possible in tests using this format.

In operation the SALIANT device is ideally suited for, screening and monitoring purposes in areas of high security or where there is a suspected presence of explosives. In addition a specific application would be for evidence collection at scenes of terrorist incidents where rapid primary analysis is crucially important.

A prototype is now near completion for market launch and available for viewing for the first time in public.

You can visit SALIANT at stand 1-A40

For more information go to www.saliant.eu or contact us at info@saliant.eu

SALIANT

Forensics Europe Expo 24-25 April 2013 7

Figure 16: SALIANT feature article

A feature press release (Figure 16) was published in the forensic trade journal, The Investigator in April 2013. The e-journal has an identified readership of around 5000 subscribers from the security sectors in UK,EU ,AU,NZ and US.

4.5 SALIANT Video

A short (approx. 4mins) film entitled '**SALIANT**: Rapid detection of trace analytes' has been prepared for assisting with dissemination post project. The video has been professionally produced and combines location filming and expert interviews to explore the current challenges of detecting small weight analytes; present SALIANT's state-of-the-art technology for rapid and positive detection of such analytes; and discuss the potential applications of such technology, particularly in relation to the forensic detection and screening of explosives.

Laboratory-based photography captures the device in action together with interviews from the developers, whilst interviews with current and potential end users captures *in-situ* where the device might be implemented.

Audience

The video is predominantly aimed at scientists and end users and care has been taken to promote the advantages and ease of use of the SALIANT technology. Consideration has also been given to ensure the technology is able to be understood by a non technical audience.

Distribution

The film is capable of being embedded across websites including www.SALIANT.eu and within presentations. A link to the video has been sent to a SALIANT specific mailing list accompanied by a press release announcing the conclusion of the project and its main findings.

The video will also be posted on the Enterprise Europe Network website (www.een.ec.europa.eu) and included within the Enterprise Europe Network newsletter.

4.6 Trade Exhibition

SALIANT attended Forensics Europe Expo 2013 at London Olympia in April 2013 as an exhibitor. The event was selected for its high profile and provided an ideal platform to promote the project to an international audience from the entire forensics sector and supply chain.

A total of over 3,000 people attended the two-day event and interest levels in SALIANT were high as exemplified by the level of activity on the stand. A total of 42 commercial leads were captured during the event for use in future commercial exploitation. The exploitation section of this report contains more details on some of these leads

4.7 Presentations and Workshops

Work from SALIANT was presented at the Warsaw Security Research Conference.

Two workshops have been held during the SALIANT project for dissemination purposes. A new product launch workshop was held during the Forensics EXPO event in London on April 2013. The event was attended by around 20 delegates including end users, academics and manufacturing suppliers.

A second workshop was held in Zilina in May 2013. This workshop was a training workshop given to around 30 invited First Responders to instruct them in the use of the SALIANT system and to gain feedback for future development.

5 Exploitation Planning

5.1 Introduction

Full commercialisation of the prototype SALIANT system will require taking the existing process for the prototype through a series of product development gateways. These will include processes such as identification of quality standards for manufacture; securing a robust supply chain of components and securing licence agreements for critical reagents. Exploitation of SALIANT results should follow the guidelines set out in the European Security Research Innovation Agenda (ESRIA) described earlier in this report.

Successful exploitation will need to ensure compliance of the SALIANT system with national regulations and existing forensic protocols. Compliance of the SALIANT system with some of the known integration issues were discussed in the results section of this report.

Successful market entry with the SALIANT system will also depend on adoption of a defined strategy and its implementation. This report outlines some potential routes to market for the system as a whole as well as its component parts with indications of anticipated timelines.

The following sections aim to summarise the market segmentation and dynamics, key competing technologies and commercial products currently available for explosive trace detection (ETD).

5.2 Market Opportunities

The Explosive Trace Detection (ETD) market is reported to be undergoing an accelerated growth period globally, owing to a combination of factors including increasing investments in counter-terror measures, US legislation that is enforcing cargo screening on passenger flights, increased security measures at critical infrastructure points amongst others[1]. The Homeland Security Research Corporation analysts have forecast a multi-billion dollar business opportunity for ETD including pre- and post-sales activities at a CAGR of 14 %.

Screening and detection of trace levels of explosives is increasingly becoming a routine practice in sectors including:

(a) **Transport** (screening in airports, ports, borders for passenger and cargo)

(b) **Military** (counter threats against military personnel, equipment and installations)

[1] Explosives Trace Detection (ETD): Technologies & Global Market – 2013-2020 (June 2013) published by Homeland Security Market Research

(c) **Critical infrastructure** (screening in power plants, national monuments, embassies etc)

(d) **VVIP and VIP security** (screening)

(e) **Forensics** to collect evidence for investigation and prosecution purposes post-scenario (e.g. after terrorist incidents

The requirements and challenges vary slightly in each of the above application area in terms of sampling, throughput required, storage and transmission of data and performance parameters required.

Several small and large companies have attempted to capitalise on the opportunities these sectors have to offer and have developed technologies and systems for ETD, with varying levels of cost, performance and features and hence, end-user benefits.

It appears that there is no single system that can offer all required performance specifications at low costs and required ease of use. A critical requirement in all of these applications is the need for very low or no false positives or false negatives (False alarms). It is clear that there is a need for complementary systems based on different fundamental technologies to add to current capability in this critical sector.

5.3 Market Analysis

ETD sensors and systems that are capable of detecting minute amounts of a variety of explosives are being deployed globally to extend and improve counter-terrorist and security efforts, enhance safety of public and critical infrastructure and for forensic purposes to obtain evidence post-scenario that could be used in court.

Most commercial systems are based on variations of Ion Mobility Spectroscopy (IMS) and other Chemiluminescence methods. Traditional canine-based detection continues to remain popular in some application areas given their ease of use and high sensitivity.

The competitor watch carried out by SALIANT concluded there is good evidence of a very active market for explosives trace detection. There are other technologies that are trying to penetrate this growing market and all new products/technologies have to demonstrate complementarity or a better performance specification as compared against the existing products and technologies.

5.4 Competitor Analysis

For SALIANT technology to compete in the screening and forensics market, the performance specifications and potential end-user benefits have to be demonstrated. The benefits and competitive advantages of the SALIANT system

were compared against other established technologies and products in an attempt to evaluate competition for the future SALIANT product

From the competitor analysis carried out the SALIANT system compared favourably and in some cases superior, to established products on the market.

Particular advantages of the SALIANT system currently include:

- High performance – positive detection of high levels can be seen by eye within 10 seconds, quantification of low levels from 2 -10 minutes.
- Large working range of explosive concentrations
- Highly convenient – minimally requiring a simple dipstick, swab and running buffer
- Inexpensive – no large instrument costs or servicing costs
- Simplicity – for use by minimally trained personnel
- Intuitive – No red line – no explosive; More red line – more explosive
- Non-destructive testing – stick may be subsequently re-analysed by other complementary means
- Stability of result – sticks are stable for archiving/legal requirements
- Complementary – to IMS and other technologies
- Adaptability – into different formats to meet end-user requirements
- Rapid visual or reader readout – with data storage/transmission
- Excellent consortium network - for future dissemination and feedback

5.5 Future Commercialisation of SALIANT

It is recognised that entry into the security market with a new technology will be a long and potentially expensive process. The tightly regulated environment combined with a mature supply chain could be expected to mitigate against significant revenues being realised in the short term. In addition the successful adoption of the SALIANT system may require adaptation to comply with forensic best practice and national regulations for each target country. The next immediate priority should, therefore, be to devise an implementation strategy and market entry process for launching the SALIANT system in single markets on a sequential basis. Raising awareness of SALIANT, to enhance adoption by end users, will be an important ongoing activity for future commercialisation that would be clearly aided by attendance at conferences and professional forums.

The associated costs for product launch into the market as described may be significant hence a viable commercial model is essential. One possibility could be to develop alliances and commercial partnerships with established suppliers to the security sector to accelerate market entry. Another possibility could be to develop a licensing strategy to offer a product manufacturing or a distribution licence to established suppliers in return for upfront payments and ongoing royalties from sales.

Near Term Opportunities

The SALIANT system in its current prototype formation has opportunities for commercialisation in the near term as a first generation product for the detection of TNT, PETN and RDX. The product would consist of the reader as developed by Reagena, the lateral flow test strip as developed by Selective Antibodies and the swabbing system based on the BASAN swab developed by DLO.

In the first instance Selective Antibodies will lead the product development in collaboration with commercial partners where appropriate to access the market.

Product Development Collaboration and Distribution

A US manufacturer and global distributor of forensic tests to the security sector, has expressed interest in a commercial collaboration to develop the prototype through to full commercial product with potential interest in acquiring distribution rights. A partnership of this type with an established supplier already accepted by the security sector would accelerate market entry for SALIANT.

Medium Term Opportunities

Air Sampling System

The air sampling system as developed by consortium partner Applikon from the programme. While not as fully developed as the simpler swabbing system this elegant device, integrated with high performance dipsticks, shows great potential in future product developments of the SALIANT system to rapidly scan post-blast scenes.

Ostendum Biosensor

Development of this product is at an early stage with clear technical challenges remaining to be met, however the Ostendum biochip sensor has the potential to perform continuous monitoring of multiple analytes using the SALIANT chemistry. Successful development of the biochip could offer a second generation of products for the SALIANT system.

Longer term possibilities

System Design Improvements

Future developments in the analysis of post blast scenes are moving towards the removal of personnel to reduce risk as much as possible. The use of robotics for taking samples is increasing and SALIANT has had preliminary discussions with an international developer and manufacturer in this area around the development of the SALIANT system for operation by robotic means.

5.6 Intellectual property

Licensing of critical reagents

Reagent antibodies used in the SALIANT project were procured under a research licence from the UK company Ploughshare Ltd with an option to convert to a full commercial licence if the commercialisation of the SALIANT system is undertaken.

Background IP

Under the terms of the consortium agreement all background intellectual property used by the project is available to the consortium partners for future commercialisation activities if required.

Foreground IP

The SALIANT programme resulted in a very significant amount of valuable commercial 'know-how'. It is intended that this be applied during the commercialisation of products stemming from the programme and for devices aimed at the rapid quantitative detection of other small molecules of interest not only in the forensic and security sectors but also with respect to human and animal health and also within the food and drink and environmental sectors. Expert opinion is being sought as to whether to formally patent outcomes of the programme.

6. Contact Information

Selective Antibodies Ltd is happy to be contacted as the point of first contact for interested parties:

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