



Technology Sector – Security

Sub Sector – Detection

Segment - Biological

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## 1. Title

Detection of Biological threats

Keywords – anthrax, ricin, virus, bacteria, toxin, nucleic acid, biomolecular, immuno-detection, biosensors, nanowires, nanotubes, terahertz, optical methods

## 2. Definition

Biological threats have been defined as those viruses, bacteria and toxins which deliberately spread can cause serious harm to humans, animals and plants. 23 bacteria, 43 viruses and 14 toxins have been reported by security agencies as threats. Table BW.1 provides a list of some of these biological threats [1].

**Table BW.1** – List of commonly cited harmful biological agents

	<b>Name of biological agent</b>
1	botulinium toxin
2	shiga toxin
3	diphtheria toxin
4	anthrax - Bacillus anthracis (anthrax)
5	ricin
6	ovalbumin
7	Staphylococcal Enterotoxin B (SEB)
8	Yersinia pestis (plague)
9	Variola major (smallpox)

## 3. Short Description

Detection of biological threats, which could be used against citizens and agricultural produce, involves the recognition of bacteria, viruses and toxins. The identification methods based on nucleic acid and immuno-based methods are used in detecting has been mentioned in the literature. These have also been used in food testing, clinical and environmental applications [2].

**Table BW.2** – A selection of detection of commonly known bacterial pathogens as cited in the journal paper [2]

	<b>Biorecognition molecules</b>	<b>Target</b>	<b>Type of assay</b>	<b>Estimated time of assay (h)</b>	<b>Sensitivity (no. of targets)</b>	<b>Specificity</b>
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Nucleic acid	pst gene probes and primers	Yersinia pestis	5% nuclease fluorogenic assay	2.5 h	3–30 cells	Very high
	Heat stable toxin gene (ST-gene)	E. coli	PCR-ELISA; color amplified PCR (CAPS)	3.5 h	270 cfu	High
	Genomic DNA; Escherichia coli	E. coli and lambda phage	Lambda phage Molecular combing, scanning force microscopy	2 h	103 cells	High
	Universal and specific 16s rRNA primers	B. subtilis, Y. pestis and E. coli	Multiplex PCR followed by gel electrophoresis	3 h	ND (5 ml of soil)	High
Immunoassays	Fluorescently labeled MAb	Salmonella typhimurium	Flow cytometry	40 min	103 cells:ml of milk & egg	High
	Fluorescently labeled PAb to 0157 antigen	E. coli 0157:H7	Antibody-direct Epifluorescent filter technique (Ab-DEFT)	3 h	104–105 cfu:g feces	High

**Table BW.3** – A selection of recognition techniques for detection of pathogenic viruses as cited in a journal paper [2]

	<b>Biorecognition molecules</b>	<b>Target</b>	<b>Type of assay</b>	<b>Estimated time of assay (h)</b>	<b>Sensitivity (no. of targets)</b>	<b>Specificity</b>
Nucleic acid	Biotinylated primers for gag gene and internal standard control	HIV-1 RNA:DNA	QC-PCR followed by amplicon capture, probe hybridization and Luminometry	4h	100	Very high quantitative
	Biotinylated primers for pol gene	HIV-1 RNA	PCR followed by amplicon capture and colorimetry	3 h	20 virions	Very high
	Primers for immediate early genes	Active human cytomegalovirus (hCMV)	In situ PCR followed by in situ colorimetry	4 h	100	High
	Primers for immediate early genes	hCMV	Nested PCR followed by gel electrophoresis	4 h	5 virions	High
	Primers and biotinylated probes	Hepatitis C virus (HCV)	PCR based, digoxigenin labeled amplicons are	3 h	10–100 virions	Very high

			captured by biotinylated probes			
	Digoxigenin labeled riboprobe targeting non-coding sequence	Enteroviruses	Dot blot hybridization and chemiluminescent detection	2.5 h	$10^4-10^5$ TCID	High
	Digoxigenin labeled Vp1 probe	Polioviruses serotypes	Dot blot hybridization and chemiluminescent detection	2.5 h	$10^3-10^4$ TCID	High
Immuno-assays	Fluorescein labeled human recombinant Fab (rFab)	Cells infected with herpes simplex virus (HSV)	Indirect immunofluorescence	2.5 h	ND	High
	Fluorescein labeled murine monoclonal Ab to HSV	Cells infected with HSV	Direct immunofluorescence	2 h indirect	ND (less than)	High
	Latex bound antibody to	Cytomegalovirus (CMV)	Latex agglutination, visual assay	10 min	$10^{12}$	High
	IgM antibody to	CMV	Enzyme immuno assay	10 min	$10^6$	High
	Antibody	HAV	Immuno electron microscopy	8 h	$10^4-10^5$ virions/ml	Moderate
	Radio labeled antibody	HAV	Radioimmunoassay	2 h	$10^5-10^6$ virions/ml	Moderate

Self assembled bilayers of  $Cu^{2+}/L$ -cysteine have been coated on gold surfaces are used to detect biological agents in microcantilever sensors. Dimethyl methyl phosphonate was used as a sarin nerve gas stimulant in these tests [3].

Label free biosensors also known as optical biosensors are based on direct measurements of a change taking place during a biochemical reaction on the surface of a transducer [4]. Ricin has been detected using optical biosensors within 30 minutes to a detection limit of 10ng/ml. The advantages optical biosensors offer is rapid screening and multi-analyte detection. The main disadvantage for these sensors is the reduced sensitivity for the rapid screening assays [5].

Portable fibre optic biosensors have been demonstrated in the detection of Staphylococcal Enterotoxin B. These biosensors have been demonstrated in the compact, light weight portable identification system. The multi-channel identification system is based on simultaneous fluorescence immunoassays on the surface of polystyrene fibre optic probes [6].

NASA has demonstrated the nanotechnology based biosensors that can be used to detect pathogens such as anthrax. These sensors are based on carbon nanofibres and the licensed technology is being commercialised by Early Warning Inc. Electrical signals are measured in these sensors to identify the presence of a pathogen. These sensors are equipped with

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microfluidics as well that provides the advantage of field testing, allowing detection within 30 minutes [7].

Aptamers are functional RNA oligonucleotides that are used for sensing biological agents. These are also DNA based. Aptamers based biosensors have been reported to be good for security applications. Aptamers based biosensors are relatively immature, in relation to immunoassays, due to the limited availability of aptamers and knowledge of surface immobilization [8]. Single walled carbon nanotubes based field effect transistors, has been demonstrated to monitor aptamers – protein affinity binding processes. These offer an advantage over immunological assays due to their small size in monitoring protein- protein interactions [9].

Array Biosensors have been used to detect targets such as staphylococcal enterotoxin B (SEB), ricin toxin, cholera toxin, mouse IgG and Bacillus globigii (anthrax spore simulant). Prototype for monitoring postal sorting machines was demonstrated to be successful. The array biosensors provide the advantage of being specific and non-destructive [10].

Inhibition of enzyme acetylcholinesterase has been the basis of detecting organophosphate compounds due to high specificity and selectivity. The development of single thiocholine enzyme based biosensor has been reported in the literature using screen printed carbon electrodes doped with cobalt phthalocyanine. The sensors were observed to be fabricated using electropolymerisation and ablation with ultrasound. Detection limits in the order of  $1 * 10^{-17}M$  were experimentally reported for dichlorvos, parathion and azinphos. In a separate experiment the same detection limit was reported for paraoxon. The biosensors were reported to have application in environmental monitoring [11,12].

Biosensors based on acetylcholinesterase functionalised carbon nanotubes have been demonstrated in detecting organophosphorus compounds. These sensors have shown excellent limits of detection (0.145 ppb), good precision, electrode to electrode stability, and reproducibility [13].

Metallic nanowires striped with gold, silver and nickel nanoparticles in a suspended format have been used to identify biological warfare agents such as anthrax, smallpox, ricin and botulinum. Each nanowire relating to a particular antibody detects a pathogen. The made advantage offered by this method of detection is the ability to have up to 100 different striped nanowires which reduce analysis time significant for multiple analytes [14].

Kane et al. have demonstrated the use of peptide bound carbon nanotubes in detecting toxins such as anthrax, and deactivating the anthrax toxin by using invisible and near infrared light. Carbon nanotubes coatings maybe applied as a thin coating on a range on surfaces [15]. SERS enhancements have been used to detect microorganisms using colloidal metal suspension [16].

Terahertz imaging has been demonstrated for anthrax stimulant bacillus cereus in postal envelopes [17]. The need for research in detection methods of biological threats was driven by the anthrax spores distribution through the postal services in the United States. Laser induced breakdown spectroscopy (LIBS) is a technique that uses light induced from a laser induced microplasma to determine the composition of the sample, based on elemental and molecular emission intensity. The main advantages of this technique are high sensitivity, selectivity and minimal sample preparation. LIBS have been demonstrated to have been effectively used in the detection of bacillus subtilis and ovalbumin. Experimental research has established the effectiveness of this method with few false positive or false negatives [18].

#### **4. State of Research and Development**

The sections give an overview of the technology development in relation to a specific technology. Fundamental Research is defined for this purpose as research with no particular goals of commercialisation. Applied Research is defined as research conducted in academia and industry directed towards a specific purpose and application. Prototype has been defined as Applied Research or Fundamental Research that has found a potential market application. Technologies that are in the field trial state are defined as those that are in the process of commercialisation, and are being tested. Deployed nanotechnologies are those that have found an early stage market. Mass Market has been defined as those technologies that have been adopted by large population and are attractive high growth markets. The scale of readiness mentioned ranges from fundamental research to mass market. The spread of development across the readiness level is an indication of various detection methods, research and development effort for different threat agents using varied biological means. A validation of their status is necessary from the economic and other technology sectors perspective. The table BW.4 below gives an overview of technology developments in relation to specific enabling technologies for biological threats detection.

**Table BW.4** – Comparative Research and Development Status for category of biological threats detection

	Fundamental Research	Applied Research	Prototype	Field Trials / Pilot plant ( Pre-commercialisation )	Deployed (Commercialised)	Mass Market
Nucleic Acid based Sensing		▪	▪	▪	▪	
Immunoassay sensing		▪	▪	▪	▪	
Optical biosensors		▪	▪	▪	▪	
Nanowire		▪	▪			
Nanotube		▪	▪			
Spectroscopy		▪	▪	▪	▪	

## 5. Additional demand for research

Specific research needs were mentioned in the literature relating to different detection aspects as follows:

- Leveraging telecommunications in sensing of biological warfare agents has been identified as an applied research need. Linking of sensors, detectors and inspection systems into a communication and management network similar to air traffic control has been suggested [6].
- Research on properties of paper and its scanning through the postal system has also been suggested to protect civilians from biological or chemical attack [19].

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## 6. Applications and Perspectives

In the expert engagement process for the technology segment, the following perspectives were observed:

- Funding research and development for detection of CBRNE and Narcotics was considered very important for society and economy of Europe.
- The most important drivers for research and development of '*detection of CBRNE and Narcotics*' were considered technological and social impact. The technological drivers relate to cost, performance, efficiency and absence of solutions. Other secondary drivers were indicated as competitive advantage in conflict situations, safety, productivity gains and regional security policy.
- The main drivers for R&D of '*biological detection*' were mentioned to be 'cost of sensors, devices and instrumentation', 'sensitivity', 'time for detection', 'size of detectors', 'mobility of detection unit', and 'accuracy of detection'. Other secondary drivers were identified as 'integration of detection platform', and 'life time of operation'
- The main barriers to research and development of '*detection of CBRNE and Narcotics*' were mentioned as 'availability of finance to early stage companies' and 'inadequate technology transfer from Universities'. Secondary barriers indicated were 'intellectual property conflicts', 'lack of tax incentives' and 'lack of supportive government policy'.
- Qualitative responses indicated to meet the challenges of 'availability of finance', EU needs to consider dual commercial use of security technology as the market was relatively smaller than US. While trends in US are towards government driven technology that is validated, EU grants are inadequate for proving technology. It was suggested that government validation of systems was necessary as laboratory systems not scaled for field use.
- The main barriers to R&D of '*biological detection*' were indicated as 'inadequate research funding', 'lack of reproducible results', 'failure in integrating devices' and 'poor detection limit'. Other secondary barriers were mentioned to be 'inadequate skilled personnel', 'lack of equipment and testing facility' and 'robustness of field trials'.
- 'Collection and sampling', 'integration of detector into monitoring unit', 'continuous operation' and 'specificity' were considered important functional requirement. Other important functionality for detection were indicated as 'sensitivity of specie being detected', 'reproducibility of accurate results', 'retaining functionality in wide operating conditions', and 'long operating life with minimum maintenance'.
- Other secondary desirable functionalities for detection were indicated as 'stability of detection material', 'reversibility', 'multifunctionality', 'signal transduction', 'minimal sample preparation', and 'low cost'. 'Reversibility' was considered relatively less important functionality.
- The application trends were mentioned as:
  - The characteristics of a detector application are mission and scenario dependent.

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- Development of portable and sensitive detection devices. There is a present lack of portable instruments with good sensing characteristics. Trend is towards miniaturising biological sensor systems.
  - Application development trend directed toward broad based technologies primarily for transportation hubs.
  - Development of nanostructured functional materials and interfaces for high performance detection of biological agents.
  - Systems integration is a gap in technology development for biological detection. Along with system issues, performance issues for sensors were also mentioned.
  - Low false positives and low false negatives are the most important application requirement.
  - Qualitative responses mentioned that getting the sample into the device, concentrating and analysing is a development challenge.
  - In-situ forensics application demand has been mentioned.
  - Lack of data sharing from field trials has been mentioned as a constraint for example by water companies.
  - Operational constraints were identified as environmental changes such as temperature, humidity and large number of interferants. Mobility of detection device, and calibration for temperature and humidity were mentioned as constraints.
  - Other operational constraints were mentioned to be calibration of measurement, skills and interpretation needed from operator.
  - Processing constraints were identified as lack of basic understanding to control nanomaterials in a precise manner.
  - Improving cost effectiveness by controlled large scale production and improve laboratory infrastructure for mass scale production.
  - Long development life cycles for applications are characterised by delivering scientific results, establishing performance and establishing cost effective performance of detection technologies.
  - Nanotoxicology was considered an important issue therefore development of appropriate risk assessment methods is necessary.
- The sensing methods for biological detection that are presently deployed are nucleic acid, immunoassays, optical biosensors and spectroscopy.
  - Methods for biological detection that are expected to be deployed between 5 - 10 years are nanowires, and nanotubes based sensors.
  - Development challenges for immunoassays were mentioned to be field practicability, durability of sensing surface, sample handling and cost. For nanotubes sensors, economical production of nanotubes were considered to a development issue to be addressed. Other development issues and critical factors for nanotubes and nanowires sensors were mentioned to be reproducibility, sensitivity, selectivity and pattern

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recognition. For spectroscopic techniques, miniaturising the system was considered to be the most challenging aspect.

- The very attractive and relatively higher growth market for biological detection was expected to be nucleic acid based and optical biosensors.
- The moderately attractive future growth markets for biological detection were expected to be immunoassays and nanowires.
- North America was considered relatively better than Europe which was considered better than Asia for fundamental and applied research, industrial technology development and commercialisation for the Detection sub-sector. While Asia was considered better for cost effectiveness for technology, EU was considered better for governmental policy for innovation.
- Qualitative responses indicated that US was considered to be far advanced than Europe in Biological detection. Korea, Taiwan, Japan and Singapore were conducting considerable research. Notable advances have been made in China. Research funding was mentioned to be dispersed and effort was ill directed. US was considered good for commercialisation but increasingly Asia is becoming better. It was mentioned that Europe had existing sensor deployment relatively better than other world regions, it lacked research and development for future leadership.
- Qualitative suggestions on improvement of capabilities were suggested as:
  - collaborative research between security agencies, academia and industry
  - encouraging tax exemptions
  - basic research to understand nanomaterials better
  - greater need for biochemical basic and applied research
  - technology transition from science to implemented demonstrators is gap that needs to be addressed.
  - creation of thematic networks to improve coordination of research and development activity
  - development of novel solution for sample collection and identification of a wide range of threats
  - creation of multinational, multidisciplinary fund for development
  - creating a centre for standardised testing for different sensors

The theme of integrated platform for detection of chemical, biological, explosive, radiological and nuclear threats was conducted at Dusseldorf in March 2009. The following outcome and recommendations resulted from the discussion:

- Technology was not sufficiently advanced to achieve single platform detection.
- An integrated modular system that focuses on Chemical, Biological and Explosive as one unit and Radiological-Nuclear detection as a separate module is a better approach.

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- One of the main weaknesses for CBRNE detection was considered to be systems integration. It was suggested that a statement of requirements to be produced taking nanotechnology into consideration.
  - Accuracy and reliability of measurement was considered to be most important characteristic. Reproducibility of measurements and operating life of sensor were considered to be poor for modular systems of detection.
  - The cost of false positives are very high, therefore operational definition should be developed on a case by case basis for a modular system.
  - The need for greater fundamental research in understanding the sensing mechanism was emphasised.
  - It was recommended that communication between materials and sensing community be improved in order to create mutual awareness of technical breakthroughs.
  - The first area of application is expected to be transportation hub for such a modular system.
  - Technology penetration and application driven by state for CBRNE detection.
  - It was recommended the technology readiness level for biological agents should be assessed on a case by case basis for the specific threat agent and medium of propagation.
  - It was recommended that sensor requirements for the EU are critically examined.

## **7. Current Situation within EU**

TERAEYE is another framework project that aims to develop an innovative range of inspecting passive range of systems based on Terahertz wave detection. The two dimensional array of detectors are expected to detect harmful explosive, biological, and chemical agents at airports, railways hubs and civilian zones. DINAMICS is a framework 6 project that aims to develop an integrated cost-effective nano-biological sensor for detection of bioterrorist activities. The project is developing a lab on a chip device that will detect pathogens in the water supply.

The following framework 7 projects have been funded by the European Commission in the Security theme that are relevant to biological threats detection:

- CBRNE related testing and certification facilities - a networking strategy to strengthen cooperation and knowledge exchange within Europe (CREATIF) was initiated early in 2009. The project aims to create a network of product testing facilities for CBRNE detection [20].
- Integrated mobile security kit (IMSK) was initiated in 2008. The objective of the project is to combine technology solutions from Detection of CBRNE, area surveillance, and check point control for additional sensitive security locations. The sensor data is expected to be integrated with communication and data module to a command centre [21].

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The following completed Preparatory Action for Security Research projects, were funded by the European Commission and are relevant to biological detection:

- European Security: High level study on threats responses and relevant technologies (ESSTRT). The support action project has provided a comprehensive overview of necessary responses to security challenges. These include technologies for detecting biological threats and hazardous materials at airports and travel hubs [22].
- The active terahertz imaging for security (TERASEC) project, that aims to develop terahertz detection. The detection of threats, explosives, pathogens and chemicals in person, luggage or post were the focus of the project. The Terahertz imaging systems were developed and evaluated in the 24 month period [23].
- Assessment of the quantity, identity, viability, origin and dispersion of airborne micro-organisms for application in crisis management tools (AEROBATICS) project aimed to develop a sensing system for biological threats detection. The project aimed to quantify the origin of micro-organism, analyse the micro-organism, and develop predictive model tools [24].
- Bioterrorism resilience, research, reaction-supporting activity promoting co-operation to assess the bio threat and organise a collective and comprehensive response for EU society and citizens bio security (BIO3R) project aims at improving preparedness for bioterrorism. It aimed to identify operational requirements, countermeasures against biological attack (detection and therapeutic) and resilience, ethical and legal issues [25].
- Biological Optical Detection (BODE) project aimed to address the necessities of developing a reliable and accurate detection tool for biological threats for stand-off applications using LIDAR. The project identified functional and operational requirements, proposed specifications, and produced a demonstrator [26].
- On-line monitoring of drinking water for public security from deliberate or accidental contamination (WATERSAFE) project aimed to use nanotechnologies in sensing and detoxification to protect drinking water systems for potential terrorist attacks or accidental spillage [27].

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