# Biological Detection System Technologies Technology and Industrial Base Study

FEBRUARY 2001

## A Primer On Biological Detection Technologies

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NORTH AMERICAN TECHNOLOGY AND INDUSTRIAL BASE ORGANIZATION

# Biological Detection System Technologies Technology and Industrial Base Study

**A Primer on Biological Detection Technologies**

# FINAL REPORT

**Prepared for the**
**North American Technology and Industrial Base Organization (NATIBO)**

**Prepared by**
**TRW Systems and Information Technology Group**

**February 2001**

## FOREWORD

In 1999, the North American Technology and Industrial Base Organization's (NATIBO) Steering Group commissioned a study of the biological detection system technologies and industrial base.

This report provides the results of this study, which was completed in December 2000. It documents:

- An overview of the goals of a detection system, the detection system process and applications
- A discussion of the biological aerosol point detector system technologies and currently fielded and projected biological detection systems
- An assessment of the biological aerosol detection technology industrial base for point detection
- An examination of detection system research and development efforts underway (including research and development being accomplished within U.S. Department of Defense, U.S. Department of Energy, Canadian Department of National Defence, private industry, and academia)
- A definitive analysis of the biological point detection system marketplace and challenges faced by companies pursuing technologies in this arena
- An assessment of what is needed to transition the technologies to market and implement it in both defense and commercial systems
- Conclusions drawn from a comprehensive review of the biological warfare agent threat, detection technology challenges, current state of biological detection systems, future requirements, ongoing research and development, technology and industrial base, program implementation/fiscal considerations, communications and testing.
- A specific set of recommendations are provided to overcome the technical, programmatic, fiscal, communications, and testing considerations in order to aid in the advancement of the most promising, cost-effective technologies for quick insertion by the defense community to achieve enhanced performance and cost savings.

This report was prepared for the NATIBO by TRW Systems and Information Technology Group, 12900 Federal Systems Park Drive, Fairfax, VA 22033.

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***BIOLOGICAL DETECTION SYSTEMS TECHNOLOGY AND INDUSTRIAL BASE REPORT***

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## *NATIBO Biological Detection System Technologies*
## *Technology and Industrial Base Study*

**A Primer on Biological Detection Technologies**

### *Executive Summary*

In April 1999, the North American Technology and Industrial Base Organization's (NATIBO) Steering Group commissioned a study of the biological detection system technologies and industrial base. This report, based on information received prior to December 1, 2000, addresses technical, business, and policy information related to biological detection technology research efforts and industrial capabilities in the U.S. and Canada. Based on this analysis, the study team reached the following conclusions and provided the outlined recommendations.

### *Conclusions*

#### *Biological Warfare Agent Threat*

- The biological warfare agent threat has emerged as one of today's foremost security challenges due to a number of reasons:

  1. The increasing availability and sophistication of biological weapons technology,
  2. The widespread proliferation of ballistic and cruise missiles,
  3. The changing global environment, and
  4. The tremendous lethality of biological agents.

- The U.S. and Canada were ill-prepared for countering the chemical and biological (CB) threat during the Gulf War, and readiness is only marginally better today.

- Biological warfare agents require relatively low levels of scientific and technological support and can be produced using common commercial processes.

- Limited financing and training are needed to establish a biological weapons program.

- Biological weapons have low visibility and can be deployed through a rather simple means of delivery.

- Biological and chemical warfare agents affect humans in different ways. Effects of exposure to chemical agents is almost immediate. But, effects of exposure to biological agents might not be manifest for several days and can affect wider areas because of increased toxicity.

- Both governments are concerned about the potential of terrorists to try to use new, genetically-engineered agents that might escape detection through current detection system capabilities and might defeat conventional methods of treatment.

- Crucial to eliminating or reducing the number of casualties and the spread of contamination is how quickly the release of warfare agents can be detected.

#### *Biological Warfare Agent Detection Technology Challenges*

- No single sensor detects/identifies all biological agents of interest. Several different technologies may be needed as components of a layered detection network.

- It is difficult to discriminate and measure biological warfare agents from naturally occurring background materials. Real-time detection and measurement of biological agents in the environment is daunting because of the number of potential agents to be identified, the complex nature of the agents themselves, the countless number of similar microorganisms that are a constant presence in the environment and the minute quantities of pathogen that can initiate infection. Potential biological agents can disguise themselves in apparently benign entities.

- Because of the makeup of biological warfare agents, approaches for detecting these agents differ somewhat from those technologies that are employed to detect chemical warfare agents. While biological agents are extremely complex and large in comparison to chemical warfare agents, they are only made up of a very limited number of unique building blocks. This means the detection systems have to either:

  1. Exploit the 2- and 3- dimensional configurations of biologics (e.g., using antibodies, gene probes/primers, and possibly chromatography),
  2. Use fairly generic detection/identification technologies like fluorescence, or
  3. Process the supra-molecular biological warfare agents into more manageable sizes to allow generic detection/identification by chemical warfare-type technologies (e.g., ion mobility spectrometry and mass spectrometry).

- The lethality of biological warfare agents heightens the requirements for detection system sensitivity, which can lead to increases in cost, size, weight and power requirements with present day technology. On a per-mass basis, biological warfare agents can be more lethal than chemical warfare agents. Hence, the farther the detector is from the agent release line or point, the more sensitive the system must be.

- There continues to be a large gap between the lethal threat aerosol concentration and the limits of detection of current equipment.

### *Current Systems*

- Biological detection technologies are in a much less mature stage of development than chemical detectors. Most available systems are point detection systems that are either in the field testing stage or still in the laboratory. Stand-off biological agent detection systems are in early stages of development and will not be ready for deployment for several years. Current biological agent detection systems are large, complex, expensive, and subject to false alarms. They can detect only a limited number of biological agents and only after exposure. Sensitivity, selectivity and durability of these detection technologies are not proven.

- Cost is a major impediment to both military and non-military adoption of biological warfare detection systems. However, funding for biological detection systems has been on the rise. Even so, the cost to the military must decrease before military users can create networks of sensors. And, the cost of these systems will need to come down substantially before domestic preparedness operations and commercial users could afford to buy the systems in the quantities that they would require to be effective.

- The small particle size of biological agents requires a complex identification process and detectors. The generic model for a biological point detection system includes a collector, a trigger, a detector, and an identifier.

- Most biological detection systems have significant support requirements, due to the use of wet chemistry and expensive and sensitive reagents. The use of expensive and sensitive reagents is a huge logistics burden on the user. Some currently fielded systems must be manned continuously by specialized personnel and identification depends on having the correct reagents.

- Current biological detection devices/systems require substantial power for operation. Some systems require the use of dedicated generators.

- Current detectors available are stand-alone systems that lack connectivity to military command and control networks. Successful integration of command and control systems with CB sensors is considered essential for the battlefield.

- No adequate means exist today to detect biological agents within containers or packages non-intrusively or remotely.

- Personnel responding to, managing or investigating a biologically contaminated scene cannot sufficiently detect, characterize, and delimit the extent of hazardous materials in the environment.

### *Research and Development (R&D)*

- The development of biological warfare agent detection and identification systems is one of the most intense research activities in defense R&D.

- Biological detection technologies research emphasis is aimed at:

  1. Improvements to biological detection and identification capability, ideally moving towards detect-to-warn capability,
  2. Emphasis on reduced weight, automation, and field-portability,
  3. Integration of components into a single, rugged system that optimizes power while retaining modularity to support upgrades, and
  4. The ability to protect valuable fixed assets such as a field hospital or airfield.

- A number of different candidate technologies are being researched for possible use in next generation detection systems, dependent upon their ease of use and level of logistical support requirements. Developing dry technologies for these systems would reduce the logistical burden.

- More investment in fast, sensitive and accurate bio-weapon detection is needed.

- Further research into sample collection and processing is required.

- Greater cooperation between military and civil authorities and a closer relationship between U.S. efforts and those of other friendly countries are needed. Military and civil R&D programs conduct R&D in similar areas as well as in support of similar user communities. They pursue many of the same capabilities, target the same types of technologies, and contract with many of the same laboratories to perform the R&D work. However, participation in formal and informal coordination mechanisms has been cited as inconsistent.

- One challenge facing the community is to ensure the effective integration of new and emerging sensor technologies into current and future detection programs.

### *Future Biological Detection System Requirements*

- Detection systems need to be deployable and supportable across the entire spectrum of military operations and for the full duration of those operations. Continuous, long term monitoring may be required for high priority fixed sites.

- Systems must have low false-positive rates.

- The ability to detect biological warfare agents in water supplies is also needed. At many of the military fixed sites, troops draw potable water supplies from uncontrolled civilian sources.

- Power components must be reduced and more efficient power sources (batteries, generators, etc.) developed/integrated into biological detection systems to reduce the size and weight of the system, to reduce supportability requirements and to increase system utility.

- Desired biological detection features include:

  - operable with minimal supporting infrastructure
  - operable in a variety of terrain
  - must interface with existing and planned command and control systems
  - robust equipment that can withstand vehicle transport and environmental extremes
  - man-portable
  - high-volume automated throughput
  - inexpensive
  - disposable or decontamination-capable
  - minimal requirement for specialized training
  - operable for long periods of time with minimal maintenance
  - long shelf-life
  - broad-ranged and able to add new threat agents rapidly
  - sensitive to civilian population susceptibility
  - low false positive alarm rates that reflect specific mission requirements
  - rapid detection and identification

- One need of future enhancements to current detection systems is to incorporate technologies that enable better characterization and portrayal of background interference for point and standoff biosensors.

- Systems capable of non-specific identification, e.g., determining the presence of bacteria, toxins and viruses by targeting generic factors, are highly desirable. Broad based detection may provide a means for detecting biologically engineered threats with signatures that are different from the agents current systems are programmed to identify.

- Improved sample collection systems for air, surfaces, water and soil are needed. DNA based detection/identification is feasible for military field detection requirements only after a sample has been collected, contaminants have been removed from the sample, and a "clean" sample (inhibitors removed) has been presented to the identification component (e.g., polymerase chain reaction, mass spectrometry). Speed of detection using DNA-based detectors could be accelerated with the development of improved sample preparation systems.

- Another needed capability is for non-intrusive detection of biological agents (e.g., screening cargo, mail, packages, etc.).

### *Technology and Industrial Base*

- The biological agent detection technology industrial base sector is primarily supported by small and medium sized companies.

- Many of these companies are in the development stages of technological maturity, with very small scale manufacturing capabilities.

- Most of the companies involved in this arena have already formed or are actively forming teaming arrangements in order to be able to fulfill requirements.

- Smaller companies are teaming with larger companies, who would act as system integrators in assembling the detection system and make use of flexible manufacturing lines.

- Companies involved in development of technologies for detection systems are not solely focused on biological detection system applications, but rather for use in a variety of commercial applications as well. A number of marketplace factors influence a company's success including, among others, its ability to:

  1. Successfully commercialize a broad range of products,
  2. Keep pace with rapidly changing technology,
  3. Remain competitive,
  4. Fund R&D programs,
  5. Manage the patent process,
  6. Protect the company's trade secrets,
  7. Capitalize on collaborative opportunities and strategic partnerships,
  8. Develop products that are in demand in the marketplace, and
  9. Invest in needed capital equipment/facilities.

- A number of companies who manufacture laboratory equipment for other markets are also tracking developments in this field, looking at the potential to tailor their technologies and/or instruments for future detection systems.

- CB detection technologies have dual use potential in a number of different fields, including pharmaceutical and medical diagnostics, and monitoring air pollution and air quality in plants, noxious fumes inside enclosed areas, and municipal water supplies.

- The biological detection arena reaps the benefits of advances in other high growth technology areas, including biotechnology, computer technology, display technology, micro electronics, nano technology, communications technology, and low level signal recovery technology.

- The military forces are not the only government entities that have detection system requirements. Detection systems are needed for first responders, the U.S. Secret Service, the Federal Bureau of Investigation, fire departments, airports, embassies, and hospitals.

- There currently is not enough demand for any single biological detection system that would allow companies to make a realistic business case decision on production. Systems developed should be based on dual use technologies because the military is too small a segment of the market.

- Both the U.S. and Canadian military forces have low inventories of some biological detection equipment.

- In the U.S., detection equipment currently fielded would not be adequate to fulfill current major theater of war requirements.

- The U.S. Department of Defense (DoD) and the Canadian Department of National Defence (DND) have striven to communicate with industry on their nuclear, biological and chemical procurement plans for the future through annual Advance Planning Briefings for Industry (U.S.) and Industry Days (Canada). Both countries' defense departments are also receptive to briefings from industry on their different technologies.

### *Program Implementation/Fiscal Considerations*

- CB defense efforts of each of the four U.S. Services are coordinated through the Chemical and Biological Defense Program, which has led to a number of Joint Service projects.

- However, each of the U.S. Services also has unique, specific requirements for biological detection systems to meet their needs. Meeting the needs of all Services using common equipment is sometimes

difficult, hampering the effectiveness of joint programs. For instance, whereas the U.S. Air Force can handle a 900-pound detector, the U.S. Marine Corps wants a detector that weighs just nine pounds. U.S. inter-service disagreements hamper the DoD's efforts to deploy advanced detectors in the field. This has contributed to a lack of preparation in the technology base.

- Canada's research arm for biological detection is centralized at Defence Research Establishment Suffield. The U.S. research efforts are more decentralized, more complex, and broader ranging. Many different research components of the U.S. government are involved in U.S. biological detection R&D. Research in this area is conducted by the four Services laboratories, as well as within Department of Energy and Defense Advanced Research Projects Agency.

- Challenges faced by the DoD and DND are the rapid turnover of promising Science and Technology products and technologies, shortening acquisition times, and lowering total ownership costs. This necessitates the need to continually track new and emerging technologies and ensure an effective technology transfer/integration process.

- The U.S. funding process is very involved and lengthy, and sometimes hampers the military's ability to move forward with a promising technology or fund a new program. The U.S. players must defend their programs through the Program Objective Memorandum process every year. This can cause fluctuations in funding of programs. The Canadian DND has a shorter, more streamlined decision process in which very few decision-makers are involved and, as such, its funding is much more stabilized.

- The U.S. spends more money than Canada to fund a number of different research programs and system development initiatives in the biological detection area. This is a reflection of the size difference between the U.S. and Canadian defense R&D budgets. Given these funding constraints, DND has made considerable progress in technology development.

### *Communications*

- There are many new players in the biological defense arena, and improvements in communication are needed. Though there is formal and informal program coordination between the agencies sponsoring R&D, it is inconsistent and does not ensure that potential overlaps, gaps, and opportunities for collaboration are addressed. The Joint Program Office for Biological Detection has cited three challenges:

  1. The ability to leverage mission requirements for Domestic, Reserve, and National Guard requirements,
  2. Overcoming the instability of Service requirements, and
  3. Leveraging international collaboration.

- Information is lacking on the military forces' operations' prioritized needs, CB defense equipment requirements and how programs relate R&D projects to these needs. The requirements process needs to be defined. Competing priorities of a very complex management and oversight bureaucracy can dilute program focus. The DoD is working to alleviate this situation and intends to submit the needed information to Congress in 2001. To accomplish this, the DoD is in the process of developing performance goals and performance measures. These goals and measures will be stated along with the development of the Chemical Biological Detection Program Strategy Guidance and incorporated into key planning, programming, and budgeting documents. A Performance Plan will be completed during calendar year 2000 and included in the next annual report to Congress. DND has published a revised concept for Canadian Forces Operations – Nuclear, Biological and Chemical Defence, and is presently maturing a concept of operations for biological agent detectors.

- Department of Energy and Defense Advanced Research Projects Agency sponsored programs do not formally utilize user requirements in planning their R&D goals. These government offices have not

instituted program performance requirements to measure program performance against desired goals, as required by the Government Performance and Results Act (GPRA). The GPRA required adherence to an overall strategic plan, explicit program goals and measurable performance benchmarks.

- Civilian biological detection domestic preparedness programs lack performance measures and measurable goals. Domestic preparedness needs are not as clearly defined and not specified in as great a detail as the military has defined their requirements. No detailed equipment performance specifications or mission and threat analyses documentation has been prepared. A 1999 General Accounting Office report stated that "rapid growth is taking place in the domestic preparedness programs for responding to terrorist attacks and public health initiatives, though no sound threat and risk assessments to establish program requirements and prioritize and focus the nation's investments has been accomplished."

#### *Testing*

- There are insufficient test sites in the U.S. to accommodate all the required testing. In fact, currently there is a backlog of testing of different detection technologies.

- The Joint Field Trial (JFT) process is being standardized between primary U.S. and Canadian test facilities. Standard test methodologies, processes and procedures are in place based on previous JFT and the tri-national Test and Evaluation Working Group work. This will allow U.S. and Canadian researchers to compare data based on the same reporting results criteria.

- Additional work must be accomplished in developing and implementing new test methodologies to appropriately test emerging point and standoff technologies.

#### *Additional Concerns*

- There are different decision-makers involved in determining military and domestic response issues. How to coordinate requirements and program initiatives between these communities and determine what role the DoD and DND should play in civilian biological defense needs is a real challenge.

- For the U.S., considering that the funding for Department of Energy and Defense Advanced Research Projects Agency R&D programs have been increasing and combined are projected to be greater than the non-medical R&D funding for DOD's Chemical and Biological Defense Program for FY 2001, mechanisms for coordination need to be established to ensure that funding is used most effectively, redundant efforts are avoided, and similar requirements are handled jointly.

### *Recommendations*

The recommendations resulting from this study are designed to overcome the technical, policy, market and testing considerations addressed in the conclusions presented above. The recommendations define specific actions that should be undertaken to foster the advancement of current biological detection system technology and fielding of systems.

Based on the conclusions reached as a result of this analysis into the technology and industrial base for biological detection systems, the NATIBO Biological Detection Technologies Working Group has outlined the following recommendations. These recommendations fall into two categories: those that address technology considerations and those that address policy considerations. These recommendations highlight a roadmap of actions that the U.S. and Canadian governments should embark upon to help ensure that the future biological detection system needs of the military forces are met.

### *Technology*

- **DoD/DND should target joint R&D and biological detection system programs of mutual interest. Full use should be made of the programs in place in both countries – the U.S. Advanced Concept Technology Demonstration, the U.S. Technology Transfer Program, the Canadian Defence Industrial Research (DIR) Program, the Canadian Technology Investment Fund, and the Canadian Technology Demonstration Program - to fast track those technologies that demonstrate best value into programs.** By jointly developing biological detection systems, interoperability and supportability can be better ensured. In addition, the military forces can develop and field cutting-edge biological detection capabilities needed now, while pooling scarce resources and ensuring that there are no unnecessary duplicative efforts. The DoD has used the U.S. programs to focus on (in the near term):

  1. Collector/Concentrators – The goal is to develop a high efficiency, low power consuming collector/concentrator capable of delivering a detectable level from a low concentration aerosol.
  2. Generic Detectors – non-wet chemistry – high performing, small, low power consuming dry detectors are key to ensuring that the military forces don't miss an unorthodox biological warfare agent attack. They are also key to reducing the overall size and logistics burden of the entire detection system.
  3. Dry Detection Technologies – optical stand off technologies like LIDAR, fusing radar signals with an intelligent warning algorithm, improving methodologies for analyzing physical aerosol signatures, miniaturizing and ruggedizing detectors, and exploiting the power of networked systems. There is a big push to examine how to integrate optical standoff with other technologies.
  4. Reagents – Antibody and gene-based identification systems are the current state-of-the-art but there is also focus on developing reagents for new and emerging threat agents and in exploiting cutting edge molecular engineering techniques to improve the current reagent sets to make them more sensitive, faster reacting and more specific.

- **Alternative concepts for biological agent detection and active defense should continue to be explored**. At present, there is no silver bullet for universal detection of biological warfare agents. No one method or technique exists today that is capable of detecting all agents. Potential alternatives to currently employed technologies, perhaps discovered through technology breakthroughs achieved as a result of research being conducted in other scientific fields, could advance the capabilities of existing systems. For example, an individual-sized air purification unit based on plasma pyrolysis could be a powerful component of an overall system of active and passive biological warfare defense.

- **Some promising technologies are being developed by small companies that do not have the internal resources to participate in the Joint Field Trials**. Funding should be established in the Government technology base to support the participation of selected small businesses in their field demonstration of potentially valuable technologies and systems. Selection criteria would need to be developed to determine what constituted a promising technology.

### *Policy*

- **Requirements and standards for biological detection systems and how these relate to R&D projects should be better defined.** More detailed information about user needs, CB defense equipment requirements, and how user needs relate to R&D projects may allow more effective coordination to be achieved. If the biological detection community had access to specific data in order to compare the specific goals and objectives of R&D projects, the researchers could better assess whether overlaps, gaps, and opportunities for collaboration exist. Performance measures could also be implemented to help track progress toward goal achievement.

- **A formal process to coordinate areas of research that are supported by multiple agencies and nations should be instated and managed in the U.S. by the Deputy Assistant to the Secretary of Defense for Chemical and Biological Defense.** This coordination process could reduce potential

redundant efforts, ensure different agency requirements/concerns are addressed, provide a mechanism to share insights on technology advances/drawbacks, and enhance opportunities for collaboration.

- **The DoD/DND should sponsor bi-annual Biological Detection Conferences.** As demonstrated by the success of the First Joint Conference on Point Detection for Chemical and Biological Defense held in October 2000 and the recent Defence Research Establishment Suffield Chemical/Biological Industry Day, these types of fora provide an invaluable opportunity for the CB communities to share ideas, discuss potential technological advances, and collaborate on possible joint opportunities. Conferences of this nature could help to foster improved dialogue between companies possessing the different pieces of a biological agent detection system as well as with the military organizations. It could prove to be a catalyst to bring electro, mechanical, optics, electronics, and bio-technology firms together.

- **The JFT process should continue to be supported/funded. Work should continue to improve existing test methodologies and procedures as well as develop new methodologies to support emerging technologies.** Improved standards will allow U.S. and Canadian researchers to directly compare data from different testing sites and analyze the effectiveness of different technologies in order to gauge what programs and technologies should be targeted for transition. In fact, it is conceivable that, in the future, with these guidelines, industry could have their technologies tested at different testing sites and their data submitted to the JFT Joint Abbreviated Analysis for analysis. The military services should take full advantage of the JFTs to objectively evaluate potential technologies for inclusion in biological warfare agent detection systems. These tests provide materiel developers with opportunities to conduct field and chamber testing on their technologies while gaining performance data early on in their programs that they wouldn't otherwise be able to afford. It is an excellent opportunity for them to showcase technologies that have great potential, but lack strong sponsorship. These reports are also open to other appropriate government agencies for their uses. The JFT process has been touted as setting the standards for domestic and international biological detection test methodologies and has been adopted by Canada and the United Kingdom, and set the baseline for the International Field Trials completed this year in Canada.

- **A bottom-up review of future biological detection requirements and operational concepts with emphasis on integration, interoperability, and operational utility should be considered.** Earlier research was focused on specific technologies like state-of-the-art power systems, collection systems, and communications and information technologies, but these were carried out without emphasis on the larger system requirements. The current point detection systems all deal with detection of agents after people have already been exposed, and the next step is medical rather than operational. Future systems should develop a "system of systems" concept that could maintain operational effectiveness in a biological warfare environment.

- **Much more emphasis and sustained, stable funding is needed over a period of time long enough to allow the DoD and DND to research new technologies, move things out of the R&D base, ensure effective command and control communications with other systems, and field them.** Heightened focus and research dollars should be devoted to the biological detection program. There is a clear need for new technologies, especially with the demanding requirements of biological agent detection and identification. Traditional hardware systems and/or immuno-assay approaches may be less effective in dealing with complex environments such as cities and populated areas. And, greater investment in technologies like state-of-the-art power systems, collection systems, and communications and information technology programs for integration into warning and reporting networks is needed. This would allow systems to be reduced in size, be more fully automated and ensure that interoperability requirements are met. Incorporation of these supporting technologies into new/advanced platforms could allow for the use of robotics, unattended ground sensors, and unmanned aerial vehicles. Key to this is ensuring funding stability. A good rapport with industry cannot be established if funding needed for a multi-year program is subject to fluctuations. Industry makes business decisions based on the total level of funding budgeted for that program. On the U.S. side especially, funding provided for a program that has suddenly been stripped has led to the disruption of ongoing industrial programs and caused friction with industry partners.

## *1.0 INTRODUCTION*

### *1.1 Background*

#### *1.1.1 The Chemical/Biological Detection System Situation*

As the most recent conflicts have shown, the threat of a biological attack is real and the ability to detect exposure to biological warfare agents early on is of paramount importance to the safety of U.S. and Canadian troops. According to U.S. Secretary of Defense William Cohen in his report, "*Proliferation: Threat and Response*",

> "America's military superiority cannot shield us completely from the NBC threat. Indeed, a paradox of the new strategic environment is that American military superiority actually increases the threat of nuclear, biological, and chemical attack against us by creating incentives for adversaries to challenge us asymmetrically."

The Quadrennial Defense Review (QDR) placed special emphasis on chemical, biological, nuclear, and other asymmetric threats. The QDR determined that chemical and biological (CB) weapons attacks were "a likely condition of future warfare" and as such, the attacks against our forces probably will occur early in a conflict.

In light of these concerns, the U.S. Department of Defense (DoD) and Canadian Department of National Defence (DND) stepped up efforts to develop and field detection systems to help protect their military forces and fixed assets. This report will address the initiatives undertaken by the two countries, the technologies they are employing, and the challenges facing them.

#### *1.1.2 The NATIBO*

The North American Technology and Industrial Base Organization (NATIBO) is chartered to foster cooperative planning and technology and industrial base program development among and between the U.S. Military Services and their Canadian counterparts. Its mission is to promote a cost-effective, healthy technology and industrial base that is responsive to the national and economic security needs of the U.S. and Canada. Formally chartered in 1987, its objectives are to:

- develop and execute technology and industrial base programs and policies
- foster policies and programs for integrating defense and commercial industrial sectors
- leverage resources
- coordinate activities and foster implementation of the resulting recommendations
- exchange data and raise issues with other bilateral committees.

To further this mission, the NATIBO has spearheaded an effort to address the challenges of advancing and maintaining technological superiority in light of reduced government research and development funding. The criteria used for selecting technologies to study through this program are:

- The candidate is a key technology area of high interest
- There is potential for both military and commercial applications
- Development and/or production exists in both the U.S. and Canada
- There is a good window of opportunity for investment and application.

Through this initiative, common areas of interest are assessed jointly, allowing participating organizations to capture the information they need cost effectively, avoid duplication of effort, and capitalize on scarce resources.

After a thorough selection process, the area of biological detection system technologies was chosen as the technology area to address through the NATIBO's technology base enhancement program. The NATIBO undertook this area of study due to concerns raised by experts in this field about the changing world threat, U.S./Canadian vulnerability to a terrorist attack, the tremendous lethality of biological agents, recognized deficiencies in existing detection systems, and the benefits reaped from timely detection.

Through the NATIBO's cooperative efforts, the U.S. and Canada have succeeded in:

- enhancing communication
- leveraging dollars
- reducing redundancy
- promoting tri-service and North American cooperation

- realizing rapid technology insertion
- commercializing technologies
- providing access to many Government agencies
- garnering high level DoD/DND visibility and endorsement of technology and industrial base initiatives.

### *1.2 Purpose*

The purpose of this biological detection technologies study is to assess the industrial base, technological maturity, level of use, utility, and viability of aerosol biological detection technologies for point detection applications. The NATIBO study assesses the state-of-the-art and future trends of the detection system technologies and its supporting industrial base, as well as the ability of industry to meet future military defense requirements. This report investigates biological detection technologies from technological, policy, financial, and effectiveness points of view and develops conclusions regarding the status of these technologies from each of these perspectives. Recommendations are presented regarding actions that the defense and industrial base communities might consider in response to these conclusions.

### *1.3 Objectives*

The objectives of this study are to provide:

- An overview of the goals of a detection system, the detection system process and applications
- A discussion of the biological detection system technologies and currently fielded and projected biological detection systems
- An assessment of the biological aerosol detection technology industrial base for point detection
- An examination of detection system Research and Development (R&D) efforts underway (including R&D being accomplished within DoD, DND, U.S. Department of Energy (DOE), private industry, and academia)
- A definitive analysis of the biological detection system marketplace and challenges faced by companies pursuing technologies in this arena
- A compilation of conclusions drawn from a comprehensive review of the current state of biological detection systems, future requirements, ongoing research and development, technological advances, communications, testing and program implementation/fiscal considerations
- A specific set of recommendations to overcome the technical, programmatic, fiscal, communications, and testing considerations in order to aid in the advancement of the most promising, cost-effective technologies for quick insertion by the defense community to achieve enhanced performance and cost savings.

### *1.4 Scope*

This study, based on information received prior to December 1, 2000, encompasses the collection and analysis of technical, business, and policy information related to biological detection technology research efforts and industrial capabilities in the U.S. and Canada. Biological detection technologies investigated were limited to aerosol point detection systems. Due to resource constraints, this report does not specifically address stand-off detection technologies, except as point of reference within the course of the broader biological detection field. Stand-off detection technologies are less mature and will most likely be addressed in the future. Within point detection technologies, the technologies were broken into the following subcategories:

- Collection and Sampling Technologies

  a. Cyclone Collectors/Samplers
  b. Virtual Impactors
  c. Bubblers/Impingers
  d. Variable Particle-Size Impactors

- Triggering and Detecting Technologies

  a. Fluorescence Particle Sizing
  b. Pyrolysis-Gas Chromatography-Ion Mobility Spectrometry
  c. Flame Photometry and Gas Chromatography
  d. Size and Shape Analysis
  e. Flow Cytometry

- Identification Technologies

  a. Mass Spectrometry

- Tandem Mass Spectrometry

b. Antibody-Based Identification

- Capillary Electrophoresis
- Ion Channel Switch
- Tissue-Based Bio-sensors
- Hand-Held Immunochromatograhic Assays (HHAs)
- SMART® Tickets
- Fiber Optic Waveguide
- Surface Plasmon Resonance
- Resonant Mirror
- Upconverting Phospor Technology
- Electrochemical Luminescence
- Threshold
- Molecular Polymer Imprints

c. DNA-Based Identification

- Polymerase Chain Reaction
- Combinatorial Peptides

d. Raman Scattering.

### *1.5 Methodology*

The biological detection systems technology and industrial base study required a clear, concise, and well-defined methodology to survey government, industry and academia effectively and compile military, commercial, political, marketplace and academic perspectives. The data collected and analyzed for this study were drawn from open literature sources, such as previously published reports, conference proceedings, journal articles, Internet home pages and other on-line sources, as well as from discussions with U.S. and Canadian representatives from industry, government and academia.

The study group's goal was to meet with a representative sample of biological detection system researchers, suppliers, end users, proponents and policy makers. Factors taken into consideration in selecting sites to visit included volume and business with the individual Services and with industry, systems produced, state of the technology, applications, and new technology development. Site visits were conducted in the U.S. Northeast, Northwest, Midwest and West Coast, and in Canada. When it was determined that an industry, university, or government site of interest would not be visited due to funding and time limitations, an extensive phone interview was conducted. Data collection guidelines were developed and used to facilitate obtaining data from all points of contact either through telephone interviews and/or site visits.

Data collected from relevant documents, World Wide Web sites, on-site facility visits, and phone interviews were analyzed and incorporated into key sections of this report: detection system overview, candidate bio-detection technologies, current biological detection systems, detection system demographics, research and development initiatives, conclusions and recommendations. This report functioned as a working document throughout the data collection and analysis phases of this study.

### *1.6 Report Structure*

In order to understand the potential of the technological industrial base, it is first important to understand the capabilities of the current systems deployed by DoD and DND. Next, it is important to understand the challenges, opportunities, and limitations of the technology industrial base. Finally, it is important to look at emerging technologies to understand what the future potential of this sector can offer. This report will look at current systems, followed by a review of the technology and industrial base, and finally, an examination of the emerging government R&D trends. From this analysis, the study team then outlines conclusions formulated after review of this analysis. This is followed by recommendations for government consideration to address the conclusions that have been derived.

Section 2 of this report provides an overview of the biological warfare agent threat facing the U.S. and Canadian militaries and their current states of readiness. It also describes the differences between biological and chemical warfare agents.

Section 3 provides an overview of the fundamentals of a detection system, outlining the functions of such a system, detection system applications and the process employed in using a detection system. It also discusses detection system elements and differences between CB detection.

Section 4 provides an assessment of biological detection system technologies, discussing how

each technology functions and providing a comparison of the advantages and limitations of each technology. It also addresses the current state of development of the technologies.

Section 5 highlights current biological detection systems fielded by the DoD and the DND or in the advanced development stage. A discussion of the goals of these systems, current limitations and future performance objectives are detailed.

Section 6 examines the biological detection system technology and industrial base demographics, providing information on dual use technology considerations, inventory concerns, and marketplace factors. Descriptive write-ups of representative companies, laboratories and academia involved in biological detection technology development are highlighted in Appendix F.

Section 7 presents an overview of the U.S. and Canadian government biological detection technologies R&D initiatives. It reviews R&D goals, technological challenges, and R&D collaboration.

Section 8 provides conclusions reached as a result of analyzing the collected data. It addresses biological agents, current systems, future requirements, and policy issues.

Section 9 pinpoints specific recommendations developed after careful consideration of the conclusions reached. These recommendations are aimed at addressing the points raised in the report and the implications of the conclusions drawn from this analysis. These recommendations are outlined to suggest potential initiatives that could help propel the advancement of key technologies and enhance current government research and collaborative efforts.

Helpful appendices are also provided to assist in reading the report.

## *2.0 BIOLOGICAL WARFARE AGENT THREAT BACKGROUND*

### *2.1 Introduction*

Biological warfare is the employment of biological agents which are living microorganisms that cause infectious diseases to produce casualties in humans or animals and damage to plants or material. Chemical warfare is also geared to producing casualties and damage, but are man-made compounds.

U.S. Secretary of State Madeleine Albright has stated that terrorism is the biggest threat to the U.S. and the world as we enter the 21st century. President Clinton told the *New York Times* in an interview following his January, 1999 State of the Union Address that he has trouble sleeping because of the threat posed by biological weapons and that he fears that a bioterrorist attack will occur. The nuclear, biological and chemical (NBC) threat has emerged as one of today's foremost security challenges due to the increasing availability of NBC weapons technology and the widespread proliferation of ballistic and cruise missiles, making long range delivery possible. Coupled with this is the changing global environment. The loss of military control and financial stability in the former Soviet Union, which was discovered to have a much more extensive CB offensive program than anticipated, resulted in the proliferation of the technology and dissemination of the scientific expertise to make and use a wide range of CB agents. The break-up of the former Soviet Union translated to the loss of one of the two world superpowers and altered the balance of power. Regional instability due to nationalistic, religious and ethnic strife is a growing threat.

Many aggressor nations believe that they need to have weapons of mass destruction (WMD) in their arsenals as an equalizer against the western alliance. These warfare agents require relatively low levels of scientific and technological support and their production can be extensions of both naturally occurring and relatively common commercial processes. Any state with a basic pharmaceutical, biotechnological, or related industry could produce basic biological agents. Many legitimate biological, agricultural, and medical techniques are dual-use technologies that could be combined to create CB agent weapons. They can be easily converted into warfare agents. The skills used to make vaccines are the same ones used to enhance the lethality of bio-weapons. Moreover, the production of these agents are hard to detect.

Biological warfare agents are increasingly viewed by potential aggressors as cost effective offensive weapons, particularly when their potential enemies have a superior conventional capability. Limited financing and training are needed to establish a biological weapons program and biological weapon production has low visibility. Biological weapons can be dispatched through relatively easy means of delivery. Small quantities of lethal biological agents can be easily obtained, concealed, transported, and released in susceptible populations. Minute amounts of some biological weapons can cause mass casualties. Experts have noted that while some claim that the right growth mediums for producing lethal viruses or bacteria are difficult to acquire, embryonated eggs, which farmers will sell by the thousand, are an excellent medium. And, while some have stated that delivery is a major hurdle to the use of biological weapons, widely available technology and equipment, such as animal infection models, dry powder drug delivery, environmental exposure test equipment, and pesticide application, all can help disseminate biological weapons. Detection of biological agents is very difficult because thousands of different microorganisms could be used in an attack. Distinguishing the biological agents from the myriad of similar naturally occurring microorganisms in the environment makes this task especially daunting.

Many people working in the biological weapons arena have stated that the use of biological weapons against U.S. forces or cities is not a question of if, but rather when. Ken Abilek, former deputy chief of the Soviet bio-weapons program before defecting to the West in 1992, said that those who say the threat is overstated are behind the times.

At present, over 20 nations have acquired the ability to produce and deliver chemical and/or biological agents during conflicts. Iran, China, North Korea, Egypt, Libya, Syria, Taiwan and Israel are believed to have active CB weapons programs, and there is particular concern that Iran, Syria, and Libya are attempting to enhance their biological weapons capabilities. U.S. Defense Secretary Cohen has also expressed

concern about the potential of Cuba to use its biotechnology infrastructure to produce biological weapons. Some have expressed the concern that terrorists could even try to deploy such warfare agents using ballistic missiles. More than a dozen countries have operational ballistics missiles, and many more have missile development programs or agreements to obtain ballistic-missile technology from others. In a report issued by the Commission to Assess the Ballistic Missile Threat to the United States, the commission unanimously concluded that countries like North Korea and Iran could produce ballistic missiles capable of striking the U.S. within five years. In addition, a number of emerging powers are also researching cruise missile capabilities.

When U.S. and Canadian forces deployed to the Persian Gulf in 1990, they found themselves ill-prepared for countering the CB threat. During the Gulf War, there were shortages of individual protective equipment, inadequate chemical and biological agent detection devices, inadequate command emphasis on CB capabilities, and deficiencies in medical personnel training and supplies. The Persian Gulf War exposed serious deficiencies in CB agent identification, defense and remediation issues. The absence of real-time biological warfare (BW) agent detection systems forced Britain, Canada, France, and the United States to deploy air samplers that collected and concentrated aerosol particles into a liquid sample suitable for testing with a small antibody based enzymatic test kit. This system took several hours to produce a result and could only determine retrospectively if a biological attack had taken place. The immediate identification of a BW attack is essential for tactical, medical and political considerations. Individuals who become sick may be the only biodetection indicator of an attack. The relatively small quantity of agent that may be required to produce widespread effects, psychological effects, and difficulties of detection and treatment, make BW agents an attractive weapon of mass destruction. Several officials have noted that, had CB weapons been used during the Gulf War, some units might have suffered significant, unnecessary casualties. Following Operation Desert Storm, DoD identified many issues and shortfalls in supporting operations in a CB warfare environment. In its 1992 report, *Conduct of the Gulf War: Final Report to Congress*, DoD identified the following requirements related to CB defense capabilities:

- Lightweight CB warfare protective clothing and defensive equipment to reduce degradation, especially in desert climates.
- Integration of CB warfare protection and cooling systems into combat vehicles and procurement of stand-alone transportable collective protective shelters for sustained operations in a CB warfare environment.
- Greater emphasis of BW defenses in DoD programs. Inadequacies exist in detectors, vaccines, and protective equipment.
- To ensure effective contamination avoidance on future battlefields, additional NBC reconnaissance vehicles and early warning of CB contamination.
- Continued efforts to replace the water-based decontamination system.
- Continued force modernization in individual and collective protection, medical support, detection, identification, warning, and decontamination systems to ensure survivability and mission accomplishment under CB warfare battlefield conditions.

DoD and DND are making strides to improve their detection capabilities. Policies have been enacted to support this thrust though progress has been slow. The Defense Departments currently have only a few detection systems that provide relatively timely response and identification from field locations and sound an alert only after an attack has occurred.

The bombings of the New York City Trade Center, the Alfred P. Murrah Federal Building in Oklahoma City, Oklahoma, and the Khobar Towers in Saudi Arabia, among others, prompted increased emphasis on the need to strengthen the U.S. federal government's ability to effectively combat terrorism, both at home and abroad. In 1995, President Clinton issued Presidential Decision Directive (PDD)-39, U.S. Policy on Counterterrorism, to address the concern that weapons of mass destruction might be used by terrorists against domestic facilities. PDD-39 requires federal agencies to give highest priority to countering terrorist use of weapons of mass destruction and, in the event that an attack occurs, developing effective mechanisms to manage the consequences. It has mandated increased funding for CB defense initiatives, including research and development funding and procurement of CB warfare detection technologies.

PDD-39, as well as subsequent PDDs related to counter-terrorism, have initiated a wave of activity related to terrorism and WMD issues among numerous organizations. These include federal agencies, state and local law enforcement, mass transit authorities, and disaster preparedness authorities, to name just a few. Given the PDD-39 mandate to respond to the potential terrorist threat, many agencies and organizations have initiated counter-terrorist programs or have purchased equipment to manage the consequences of a terrorist attack using NBC materials. Since 1996, the number of federal programs and initiatives to combat terrorism has grown significantly. According to Office of Management and Budget (OMB), funding has increased from about $6.5 billion in Fiscal Year (FY) 1998 to about $10 billion for FY 2000.

### *2.2 Current State of Readiness for Chemical and Biological Warfare*

In a report issued by the Commission to Assess the Organization of the Federal Government to Combat the Proliferation of Weapons of Mass Destruction, the commission called the U.S. government unprepared to prevent or cope with a NBC attack. The report concluded that the government's current efforts both to prevent the spread of NBC weapons and to cope with the possible use of such weapons are disorganized. Research on foreign efforts to produce CB agents is fragmented among the Central Intelligence Agency, the U.S. Army and DOE Laboratories. Many separate government agencies have overlapping jurisdiction over aspects of the problem. The U.S. House and the U.S. Senate also have a number of committees with oversight and budgetary responsibility for nonproliferation programs (ten in the House alone) and that Congress calls for a number of reports on NBC weapons issues (112 separate reports were listed in the commission's report). And, despite the expenditure of several billion dollars since the 1991 Gulf War, the vulnerability of U.S. troops to chemical and biological weapons has only increased.[1]

In testimony before the Subcommittee on Oversight, Investigations, and Emergency Management, Committee on Transportation and Infrastructure, Mark E. Gebicke, Director of National Security Preparedness Issues for the National Security and International Affairs Division, stated that, with the proliferation of federal programs and initiatives to combat terrorism, there is the potential for duplication and overlap among these programs. Several officials have pointed out that they believe these programs have led to a fragmented and possibly wasteful federal approach to combating terrorism, and that multiple equipment programs were causing frustration and confusion, resulting in further complaints that the federal government is unfocused and has no coordinated plan for dealing with this issue.

On 9 June 1999, Mr. Gebicke noted in testimony before the Committee on Veterans' Affairs that there are deficiencies in doctrine, policy, equipment and training for the defense of critical ports and airfields. He stated that "DoD's doctrine and policy are inadequate regarding responsibility for the CB defense of overseas airfields and ports critical to the deployment, reinforcement, and logistical support of U.S. forces in the event of a conflict. As a result, questions are unresolved regarding the provision of the force structure and equipment needed to protect these facilities."[2]

Experts at a recent conference, "Emerging Threats of Biological Terrorism: Recent Developments," sponsored by the Potomac Institute and George Washington University, stated that it is no longer appropriate to think of national defense as something only done across an ocean. In order to protect the country, the conception and implementation of national security must change. They called for greater cooperation between military and civil authorities, closer relationship between U.S. security forces and those of other countries, and more investment in fast, sensitive and accurate bio-weapon detection technology.

According to the Canadian Security Intelligence Service's 1998 Public Report, Canada and Canadians are not primary targets of terrorist groups; however, proximity to the United States, a common border, large expatriate communities and a healthy economy draw representatives of virtually every terrorist group in the world to Canada. A large part of terrorist activity in Canada is related to on-going conflict abroad. Logistical support for terrorist acts in other parts of the world has been provided on Canadian soil and support networks in Canada have provided terrorists with safe-haven and transit to and from other countries, including the United States. There are several reasons for this. Canada's long

border and coastlines, and comparative wealth as a source of technology, equipment and funds appeal to terrorist groups. As with other democracies, Canada's openness and respect for individual rights and freedoms preclude the suppression of terrorism by ruthless methods and the open nature of Canadian society makes Canada particularly vulnerable to terrorist influence and activities within expatriate communities. Canadian officials have stressed the need to improve their detection and response capabilities to CB agents in response to this terrorist threat.

As cited in the Center for Counterproliferation Research study, "*The NBC Threat in 2025*", the following findings underscore the importance of shoring up the detection capabilities of the U.S. and Canada:

1. The increasing utility of unconventional delivery of NBC may require a fundamental reassessment of how the U.S. defends against the NBC threat. NBC weapons can be extremely lethal and are easily concealable and transportable weapons' packages. Civilian population centers and military facilities are attractive targets due to the perceived vulnerability of these targets.

2. The growing prospect of use (or threat of use) early in a conflict may require major changes to U.S. doctrine, force design, planning, and training. The appeal to adversaries of using NBC weapons against countries which have overwhelming conventional capabilities are numerous. Using such weapons (or threatening use of these weapons) could deter the country (ies) in intervening in a conflict, by demonstrating the potential costs and losses that could be incurred by such a venture. Adversaries may see these weapons not as instruments of last resort, but rather as weapons of choice to gain political, psychological or military advantage.

3. The expanding capability for long-range delivery will deny the U.S. a homeland sanctuary, making essential both missile defense and emergency response capabilities.

4. The unique challenges NBC weapons pose for coalition warfare will affect the way the U.S. conducts war in the future.

5. Biological weapons could well become the "weapons of choice", because they are cheap, easily produced and concealable (and will in all likelihood become more so in the future due to advances in biotechnology). Small quantities of biological warfare agents can be extraordinarily lethal. In addition, these agents do not require sophisticated delivery means. Generally, they can be released through aerosolization, which would allow for delivery via crop dusters, sprayers, and even pocket-sized atomizers. Information on development and dissemination of BW agents is readily available. And, since these weapons are easy to conceal and release, it would be difficult to detect the onslaught of such an attack.

6. Deterrence is becoming a two-way street. Traditional deterrence based primarily on punishment and retaliation may become problematic, requiring a strategy of deterrence by denial.

### *2.3 Historical Perspective*

Biological weapons have been used during warfare in a limited number of cases. In the 14th century, Tartars catapulted dead bodies over the walls of Kaffa in an attempt to introduce plague. During the French and Indian War of 1754-1767, the commander of the British forces in North America allegedly suggested using contaminated blankets to introduce smallpox in Native American populations sympathetic to the French. Germany is believed to have used biological weapons during World War I to infect livestock in a number of countries. Japan attacked eleven Chinese cities with biological agents during World War II, contaminating air, water and food with Bacillus anthracis, Vibrio cholerae, Shigella, Salmonella, and Yersinia pestis-infected fleas.

Britain, the U.S. and the Soviet Union built upon the Japanese experience with biological weapons and engaged in sophisticated biological weapons programs after World War II. The focus of these efforts was on developing a limited number of biological agents and on aerosol delivery to infect the largest possible number of targeted individuals.

The U.S. ended its biological weapons program in 1969 by a unilateral declaration of President

Nixon. The Biological and Toxin Weapons Convention, an international agreement, was put in place in 1972 prohibiting the production and retention of stockpiles of biological weapons. Even so, some nations have continued to develop a biological weapons capability, which some officials have referred to as the "poor man's nuclear arsenal". And other nations have never committed to this agreement. The ramifications of this fact were underscored by the 1991 discovery of the extensive biological weapons program of Iraq and the 1992 statement by Boris Yeltsin admitting to a clandestine massive biological weapons program in the former Soviet Union (at least 30,000 people working in as many as 50 research and production facilities) in violation of the convention.

Unlike the Chemical Weapons Convention, the Biological and Toxin Weapons Convention has no accurate verification mechanism for ensuring compliance. Without such compliance measures in place, there is no way to prevent nations from carrying out massive biological weapons programs. Maj. William King, who serves as a war planner for the U.S. Eighth Army in Korea and recently wrote a paper on the vulnerability of the U.S. Army to biological attacks, was quoted in October 18, 1999 edition of "*Inside the Army*" as saying, "Unless the international community takes prompt steps to bolster the Biological Weapons Convention or the means to deter the proliferation of biological weapons, biological weapons could be to the 21st century what nuclear weapons were to the 20th century."

### *2.4 Biological Warfare Agents*

Chemical warfare agents and biological warfare agents differ significantly in composition, complexity, and environmental behavior. Biological and chemical warfare agents affect humans in different ways. The effects of exposure to a chemical agent like sarin are going to be virtually immediate. Because they take effect more rapidly than biological toxins, chemical agents are considered better weapons on the battlefield.

The effects of biological agents, such as anthrax or botulinum toxin, might not manifest for several days. At first, the symptoms may seem innocuous and flu-like. This delay makes it difficult to trace the release point and enables the effect to range far beyond its initial release point since victims are likely to interact with other people prior to realizing they are ill. As a result, it may initially be difficult or impossible to know exactly where or when an individual was exposed or to identify an area to cordon. Decontamination and quarantining of areas, in most cases, would not help. Unlike chemical weapons, which disperse over time, biological agents may grow and multiply over time. Anthrax can remain active in the soil for at least 40 years and is highly resistant to eradication. And, infected individuals would have had plenty of time to travel to various cities before anyone knew they were infected.

There are five types of biological agents:

- Bacteria – Bacterial agents, such as anthrax, can cause diseases in human beings and animals by means of invading the tissues or by producing poisons. Bacteria are sensitive to antibiotics.
- Rickettsiae – Rickettsiae are organisms similar to bacteria with some viral properties. Rickettsiae are sensitive to antibiotics.
- Chlamydia – Chlamydia are organisms similar to bacteria, but like viruses, require host cells for multiplication. Chlamydia are sensitive to antibiotics.
- Viruses – Viruses are the simplest type of micro-organisms which lack a system for their own metabolism and depend on host cells; thus, viruses are parasites which cause disease by damaging host cells. The host cells can be from human beings, animals, plants or bacteria. Some of the viruses ranked by the defense departments to be of the highest concern include smallpox and ebola. Viruses are not sensitive to antibiotics.
- Toxins – Toxins are substances of natural origin produced by an animal, plant or microbe which can cause significant illness at levels much lower than the level required for lethality and are thus militarily significant in their ability to incapacitate personnel. Some common toxins are botulinum and ricin. Toxins are not sensitive to antibiotics.

According to a *Washington Post* article, "many biological agents could be used to make weapons."[3] But most experts agree that only a limited number of well-known biological agents can cause widespread illness and death. Among them are pathogens that cause anthrax, smallpox,

plague, botulism and viral hemorrhagic fever. The effects on humans of each of these agents is noted below:

- **Anthrax** – Studied for weapons use since the 1940s. Bacteria can survive dry or cold conditions as spores. Rapid onset. Produces a number of toxins that act to break down proteins and cause cell destruction, leading first to internal bleeding in space between lungs. Death often occurs within 24-72 hours after symptoms appear, typically from the inability to breathe, blood poisoning or infection in the space around the brain. No cure, Vaccine exists, but not widely available.
- **Smallpox** – Travels from lungs to lymph nodes to numerous organs and skin. Sudden onset of muscle pain, fever, vomiting, and spasms, then severe pustular rash. Death in 30 percent of unvaccinated persons from overwhelming infection. Survivors usually deeply scarred. No cure. Vaccinations, once nearly universal, have stopped.
- **Plague** – Studied as a weapon since 1942, inhaled form produces pneumonic plague, a severe infection of the air pockets in the lungs, typically marked by blocked sputum and rapid deterioration. Death occurs from inability to breathe and overwhelming blood infection. Can be controlled with antibiotics in very early stages.
- **Botulism** – Toxin is one of the most lethal substances known to science: $1/10^{th}$ of one-millionth of a gram can be fatal in humans. Shortly after exposure, victim suffers vomiting, abdominal pain and general weakness. Toxin inhibits a key neurotransmitter called acetylcholine, halting nerve signals to muscles. Paralysis ensues. Death often by inability to breathe. Antitoxin available. Experimental vaccine only.
- **Hemorraghic fever** – Various viruses attack small blood vessels, breaking down the walls, increasing permeability and causing uncontrollable internal bleeding. Initial flu-like symptoms followed by massive hemorrhage in mucous membranes, skin and internal organs. Coma ensues, and death often follows shock – a profound drop in blood pressure. Fatality as high as 90 percent for some viruses. Therapy available only for a few.

To illustrate the point about the lethality of biological weapons, U.S. Defense Secretary William S. Cohen held up a five-pound bag of sugar on national television a couple of years ago to dramatize how, with an equivalent amount of anthrax and you spread it with the right kind of temperatures and wind conditions, a terrorist group could eliminate over half of the population of Washington, DC. The Chairman of the Joint Chiefs of Staff considers anthrax to be the greatest biological weapons threat to U.S. military forces. After a three-year study into this threat, the DoD, acting upon the recommendation of the Secretary of Defense, announced plans to vaccinate all U.S. military personnel against anthrax. Some experts have speculated that terrorists might try to use new, genetically-engineered agents designed to defeat conventional methods of treatment. They could capitalize on advances in biotechnology and genetic engineering to modify microbial agents at a molecular level to bring about disease in different ways. Many bioengineering companies now sell all-in-one-kits to enable even high school students to perform recombinant DNA experiments. The availability of free on-line gene sequence databases and analytic software over the Internet further aids researchers interested in attaining this capability. Thus, relatively benign organisms can be transformed to cause harmful effects and can defeat conventional treatment. The development of these new agents can hinder effective detection, identification, and early warning of biological warfare attacks.

### *2.5 Aggressor Profiles*

The Memorandum of Understanding (MOU) Cooperative Program on Chemical and Biological Defensive Materiel Planning Guide for Commanders, which is comprised of U.S., Canadian, and United Kingdom representatives, cited five general types of aggressors who might possess biological weapons:

- **Global Adversary** – A global adversary would possess near parity with the friendly forces in economic strength and technology.
- **Emerging Global Adversary** – An emerging global adversary would be a dominant regional power with an advanced economy and technology base. This type of adversary might aspire to achieve regional hegemony and deny friendly force influence.

- **Regional Adversary** – A regional challenger with significant advanced economic and technology sectors and a modern professional military could seek to establish regional dominance and control, and to deny friendly force influence.
- **Rogue States** – Rogue states have generally not been defined by levels of capability, but by their willingness to act outside of prevailing norms of state conduct. Biological weapons in the hands of rogue states may be especially threatening since such states may not be influenced by traditional deterrence calculations.
- **Non-state Actors** – Non-state actors act outside of the boundaries of established state authority. They could have increased access to critical weapons and weapons technology, which could be seen as providing high payoff for achieving political results through intimidation, disruption and coercion.

The MOU noted a number of different objectives for which different groups might use biological weapons, including the following:

- **Defeat the friendly forces** – the adversary's goal in this case would be the classic military victory.
- **Defeat friendly forces in a region** – the goal would thus not be the defeat of friendly forces, but the withdrawal or significant reduction of friendly force presence and power in a region.
- **Prevent defeat by friendly forces** – the goal here would be to return to pre-conflict status quo.
- **Disrupt friendly forces** – This would entail preventing effective employment of friendly forces and coalition political and military power.
- **Deter friendly force intervention** – the purpose of this would be to raise the risks and costs to the friendly forces in order to convince the friendly forces not to act against it.
- **Punish, take revenge on, or intimidate the Allies** – Biological weapons may be used by adversaries in ways designed not to defeat or deter friendly forces, but by punitive use to inflict unacceptable casualties and damage on friendly forces and civilians.

Military and intelligence experts believe that the greatest threat to the U.S. from weapons of mass destruction is posed by terrorist groups or individuals, because nations that employed such weapons would face disproportionate retaliation. Terrorists groups are not bound by these same constraints or morals and are generally motivated by different factors. The National Defense Panel has stated that concern over the threat of retaliation from the U.S. nuclear forces would not serve as much of a deterrent for terrorists who seek to coerce or punish the U.S. or its allies. Retaliation against independent freelance groups is much more difficult because finding appropriate targets - going straight to the source - can be a problem. Hostile governments are behind some terrorist acts that are perpetrated by terrorists groups, sponsoring and funding them to commit the act, i.e., the Lockerbie incident. But, discovering who is responsible for an attack is more difficult now than in the 1970s and 1980s, because terrorist groups are less likely to brag about such acts today. And, linking these groups to a specific government with financial ties to or control of terrorist groups is challenging.

Of course, the adversary has a number of different employment options to consider when weighing how to achieve his purported goals, including:

- Threatening use
- Demonstrating the capability to employ biological weapons
- Employing biological weapons in non-lethal ways
- Using biological weapons against a variety of targets for tactical advantage
- Using biological weapons against theatre targets, both military and civilian
- Using biological weapons in a strategic sense against the friendly force homeland.

Potential targets of an adversary include combat units, military command and control, military logistics, civilian infrastructure, leadership and political targets, and civilian population.

### *2.6 Threat Scenarios*

Typical scenarios that armed forces could face include the following:

- Attack on troops using chemical or biological agents loaded into cluster munitions in order to cause extensive and immediate casualties

- Release of biological agents from spray tanks using agricultural sprayers mounted on low flying aircraft upwind from intended targets such as air bases, command headquarters and field hospitals
- Incidental release of agent which was not destroyed in the attack on an agent production facility
- Deliberate poisoning of water supplies by guerillas using infectious agents.

In the civilian world, the scenarios that federal agencies expect include the following:

- Deliberate acts of terrorism on public facilities (Tokyo subway)
- Poisoning of food or water supplies in populated areas (Rajnasheesh cult in the state of Oregon).

### *2.7 Detection System Goals*

How quickly the release of CB warfare agents are detected is crucial to eliminating or reducing the number of casualties and reducing the spread of contamination. The importance of timely detection can be underscored by recent modeling studies, which have estimated that up to 70% of deployed forces become casualties during an attack in the absence of early detection and warning. With timely detection and warning, this number is reduced to five percent or less of deployed troops becoming casualties, and the requirements to wear protection is reduced.

The goals of a detection system include the following:

- Detect agents in time to warn, protect and minimize the number of casualties. Vaccines are not available for all potential threat agents because of the large variety of potential agents, the ability of aggressors to alter the properties of the agents to reduce the effectiveness of vaccines, and the lack of commercial drivers for BW agent vaccine development. Since pre-treatment is not an option in all cases, DoD/DND <u>must</u> have detection systems to warn of attack to allow personnel to don protective clothing.
- Identify the agent in time to initiate medical therapies on casualties.
- Collect samples for independent verification.
- Monitor the levels of agents during or after an attack to ensure that the concentrations in the air are safe before defensive measures are discontinued.
- Present detection information to the command and control system in a format suitable for downwind hazard prediction and consequence management.

The nature of a chemical attack differs significantly from a biological agent attack. As noted earlier, for chemical agents, physiological responses to exposure occur quickly. For example, exposure to toxic levels of nerve agent elicits severe physiological responses within a minute or two that can result in death or incapacitation in spite of subsequent treatment. To prevent death and reduce the effects of exposure, therapies must be administered within a few minutes. Therefore, detectors co-located with personnel must be able to provide real-time warning of exposure to allow effective treatment of casualties [identify to treat]. For biological agents, physiological responses generally are much slower so there is more time to treat casualties. For example, the onset of symptoms after exposure to toxins is a few hours or longer, and to infectious organisms is at least a day. However, for many potential agents, such as botulinum and smallpox, there are limited treatments. Prevention of exposure is very important, and treatment of exposed individuals should begin within a few hours to maximize the benefit. As a result, detectors co-located with personnel must detect biological agents in real time to minimize exposure, and identify them within an appropriate time interval to allow treatment schemes to be put in place for exposed individuals.

Real-time detection and measurement of biological agents in the environment is challenging because of the number of potential agents to be distinguished, the complex nature of the agents themselves, the myriad of similar microorganisms that are a constant presence in the environment and the minute quantities of pathogen necessary to initiate infection. Potential biological agents can disguise themselves in apparently benign entities. In addition, biological agents must be detected against the overwhelming background of natural bio-organisms. The background battlefield environment has a 100,000 to one million times greater concentration of contaminants than the target biological agents. In addition, there is

extensive normal biological material in the background environment. Even tests conducted at Dugway Proving Ground and Defence Research Establishment Suffield (DRES) (the U.S. and Canadian testing grounds for biological detection technologies, respectively) are limited, as both those locations have relatively pristine environments. Testing in Washington, DC revealed background particle levels about ten times higher than those in either Dugway or DRES due to pollution and human activity.

On a per-mass basis, BW agents can be billions of times more effective than CW agents. This has implications for the design and sensitivity of the detection system used to warn of an attack. The farther the detector is from the agent release line or point, the more sensitive the system must be. With existing detection technologies, increased system sensitivity requirements translate directly into increased system size, weight and power requirements, and cost.

## 3.0 DETECTION SYSTEM OVERVIEW

### 3.1 Functions of a Detection System

For the purposes of this study, we shall consider biological agent detection systems to be part of an integrated chemical/biological/radiological detection and warning system. As such, it becomes part of the command and control system and part of the medical treatment system. The broad diversity of potential threats demands an integrated system to provide the best overall response, both active and passive.

The overall goal of a detection system should be to detect biological agents in sufficient time to warn, protect and minimize the number of casualties among those who would be exposed to the agents. The best defense would be to avoid exposure, which implies the need for, at least, near-real time detection.

The second function of a detection system would be to identify the agent in time to initiate medical treatment on casualties. Whereas chemical agents act very quickly, the onset of toxic effects from biological agents is slower (because the agent must "grow" and infect the new host) and the variety of treatments is greater. In general, a positive identification of the agent in less than 30 minutes will allow the correct treatment to be initiated, provided the facilities are available.

The third function of a detection system should be to collect and capture a sample for independent verification. Such independent verification would normally take place in a laboratory environment.

The fourth function of a detection system would be to monitor the levels of agents during and after an attack to ensure that the concentrations of the agents in the air are safe before passive defensive measures are discontinued.

The fifth function of a detection system would be to provide detection information to the command and control and medical response functions. The content of the warning should provide a suitable basis for downwind hazard prediction and crisis response management. The timeliness of the warning is particularly important because adequate response preparation is the best defense. This becomes especially important in civil defense situations where resources and training may be lacking.

### 3.2 Detection System Process and Applications

#### 3.2.1 Stand-off Detection

Stand-off detection systems may be fixed, portable or mobile. Mobile systems would be meant to move with the forces. Fixed or portable systems would be intended for more permanent or semi-permanent installation. The useful range of a stand-off detection system would be sufficient to detect the aerosol cloud of agent sufficiently early, depending on wind conditions, to provide tactical warning to exposed troops.

Clearly, any system that requires the use of a sample will not qualify as a stand-off system. Therefore, a stand-off system must be able to detect and discriminate among the observable phenomena associated with the various agents. The observable phenomena may occur naturally or may result from external stimulation or excitation, such as a laser.

Thought has been given to using unmanned aerial vehicles to provide stand-off detection capability. Although the size and weight of current systems argues against employing them in small Unmanned Aerial Vehicle (UAV), some progress is being made. In December 1999, the U.S. Navy UAV Executive Steering Group prioritized missions for small UAVs and included CB agent detection as one of four practical missions. The Secretary of the Navy established a Small UAV Initiative to define these requirements and rapidly deploy advanced technology systems.

#### 3.2.2 Remote Point Detection

Remote point detection systems may be manned or unmanned. The concept is to surround a high value facility with deployed sensors, that may or may not be networked, to provide a better picture of the size and concentration of an agent cloud. The location and geometry of the sensor deployment would take into account both the geography, the prevailing winds, and the nature of the threats.

A manned point biological agent detection system, the Biological Integrated Detection System (BIDS) non-developmental item (NDI) and Preplanned Product Improvement (P3I), has been fielded and deployed by the U.S. Army. The systems were developed by the Joint

Program Office for Biological Defense (JPO-BD) in response to an immediate requirement for a field portable biological agent detection system. The more advanced version, the P3I BIDS, was fielded in first quarter FY2000. DoD's first joint and fully automated biological detection program, the Joint Biological Point Detection System (JBPDS), is currently under development. Canada has developed its own field portable biological agent detection system, the Canadian Integrated Biological Agent Detection System (CIBADS), and has also produced the next generation of this system, the CIBADS II. More details on these systems are provided in Section 5. Other countries are presently developing similar systems.

Remote point detection systems are versatile. Deployed up-wind from a high value fixed asset, they are used to provide advanced warning in order to avoid exposure. Small, lightweight systems also can be used as on-site detectors and can be mounted in vehicles for reconnaissance applications. The more advanced automated systems also are ideal for monitoring the level of agent remaining after an attack and for providing information that can be used in downwind hazard prediction.

#### *3.2.3 Point and Personal Detection*

Point and personal detection systems will emphasize light weight, low power and low cost. Few portable biological agent detectors exist, although many programs to develop them are under way.

Systems that combine CB agent detection and automatic alarming and messaging are being developed. None are small enough or inexpensive enough to meet the cost requirements for large-scale deployment. Only a few approach the size and power requirements.

Point and personal detectors are designed to be issued in larger numbers and provide information on the level of contamination by agents in a localized area. They also provide immediate warning to the wearer or the local group of personnel.

#### *3.2.4 Reconnaissance*

Reconnaissance systems combine CB agent detection and are typically mounted within a self-contained, high mobility vehicle. Such systems allow more sophisticated environmental sampling techniques than point and personal detection systems. The Joint Light NBC reconnaissance system is under development and will be capable of performing both CB reconnaissance.

Unmanned vehicles are being studied as a means of conducting CB reconnaissance without placing personnel at risk. Unmanned aerial vehicles have limited payload capability and may be difficult to employ in close proximity to troops, the areas of most concern. Unmanned ground vehicles would be better described as remotely operated robotic vehicles, and their utility is undetermined at this point.

### *3.3 Detection System Process*

#### *3.3.1 Detection System Elements*

An operational biological detection system may be comprised of, at the front end, a sampler/collector, followed by a trigger to activate the system, non-specific or specific detectors to classify the potential agent and finally, an identifier to provide specific identification. The back end process would be performed by an information management system to record, alarm, warn and message information to the command and control system.

Several types of samplers/collectors have been evaluated for biological agent detection. The principal differences between collection for biological detection and other types of aerosol or particulate sampling are:

- Biological sampling is normally targeted at living organisms, so the sampling technique must preserve and not harm the collected sample,
- Most biological detection and presumptive and confirmatory identification technologies require a liquid sample, so the collection must be from an aerosol or particulate onto a liquid,
- Since response time is a critical factor, the liquid sample must be highly concentrated and available for analysis rapidly.

#### *3.3.2 Generic Point Detection System*

The generic model for a point detection system will include four elements; a collector, a trigger, a detector, and an identifier. Section 4 will

discuss the specific technologies involved in each element of a detection system. This section will discuss in general terms the application of the various technologies to the system elements. Table 3.1, Generic Point Detection System Elements, provides an overview of this description. The generic system assumes that the medium for transport of the biological agent is an aerosol cloud. It does not, for instance, consider water-borne biological agents.

A collector is needed to concentrate the aerosol since an extremely low airborne concentration of BW agents can constitute a serious threat. The collector is generally a concentration and retention device that collects and preserves samples for further analysis.

The trigger component provides non-specific detection of the presence of possibly harmful biological material. The trigger component should give a rapid indication of the likely presence, but not the identity, of biological material and normally bases this indication on a change in the background conditions.

The detector component determines the presence of categories of biological agents, but may not provide sufficiently specific information on which to base protective or treatment decisions.

The identifier component, as the name implies, identifies the specific BW agent to the degree necessary to allow commanders to initiate appropriate protective measures.

Table 3.1 provides an overview of the technologies involved in a biological warfare detection system and where those technologies can be applied in the sense of the overall system.

**Table 3.1, Generic Point Detection System Elements**

| Collector | Trigger | Detector | Identifier |
|---|---|---|---|
| • Cyclones<br>• Virtual Impactors<br>• Bubblers/Impingers | • Particle Size<br>– Aerodynamic Particle Sizing (APS)<br>• Particle Counting | • Bio-luminescence<br>• Fluorescence | • Mass Spectrometry<br>• Antibody-based<br>– Capillary Electrophoresis<br>– Ion Channel Switch<br>– Tissue Based Bio-sensor<br>– HHA/SMART® tickets<br>– Fiber Optic Waveguide<br>– Surface Plasmon Resonance<br>– Resonant Mirror<br>– Up-Converting Phosphor Technology<br>– Electrochemical Luminescence<br>– Threshold<br>– Molecular Polymeric Imprints<br>• DNA-based<br>– Polymerase Chain Reaction<br>– Combinatorial Peptides<br>• Raman Scattering |
| | **Combined Trigger/Detector**<br>• Fluorescence and Particle Sizing<br>• Size and Shape Analysis<br>• Pyrolysis/Gas Chromatography/Ion Mobility Spectrometry<br>• Chemical Luminescence<br>• Flame Photometry/Gas Chromatography<br>• Flow Cytometry | | |

### *3.3.3 Generic Stand-off Detection System*

A generic stand-off detection system differs greatly from a point detection system. The purpose of a stand-off detection system is not so much to identify the specific biological agent but more to determine the release and location of something that very well could be a threat agent. Therefore, a stand-off detection system will be non-specific and will differ greatly from a point detection system.

The general procedure for active stand-off detection is to interrogate an aerosol cloud through external energy sources, such as an IR laser. Particulates will reflect a portion of the laser energy back to a receiver, providing indication of a potential release. A UV laser could also be utilized to further interrogate the cloud. This would provide a generic discrimination based on the fluorescence of the biological material within the cloud (biological vs. non-biological). There are no passive BW stand-off sensors currently available.

Table 3.2 provides an overview of the technologies involved in a biological warfare stand-off detection system

### *3.3.4 Differences Between Chemical and Biological Identification*

The differences between chemical detection and biological detection are as basic as the differences between chemistry and biology. Chemical reactions can be made to occur quite rapidly, and thus chemical detection can occur in near-real time. Biological identification depends on the characterization and differentiation of living organisms, a process that can be orders of magnitude longer.

Perhaps the most important difference is the identification times. Chemical detection is automatic and fast, while biological agent identification detection is much slower. Among the concerns with biological agent identification are the limited number of biological agents for which detection antibodies and antigens, have been developed. Efforts are currently underway to develop antibodies for the full spectrum of threat biological agents, but this effort is still years from completion. As technology advances, the qualitative gap between CB agent identifiers will narrow and biological detection systems will become more user-friendly, faster, smaller and able to detect a larger spectrum of threat agents.

**Table 3.2, Generic Stand-off System Elements**

| **Excitation** | **Scanning/Collection** | **Detection** | **Identification** |
|---|---|---|---|
| Active<br>• LASER<br>- Ultraviolet<br>- Near-Infrared<br>- Far-Infrared<br>• Microwave<br>• Millimeter wave | • Spectral | (Processing) | (Desired Capability) |
| Passive<br>• Background Radiation | • Spatial | | |

## *4.0 CANDIDATE BIOLOGICAL DETECTION TECHNOLOGIES*

This section provides an executive-level description of a set of candidate technologies that are under consideration for incorporation into previously fielded systems within the next two to three years. These technologies were identified by the NATIBO Technical Advisory Panel as having the highest potential for maturing sufficiently within the necessary time frame so as to warrant serious consideration for further development. These criteria exclude other technologies that, while having promise, are not viewed as being sufficiently mature to warrant their consideration at this time. This does not, however, preclude their consideration in the future.

Bio-detection is a relatively complex process. To examine and assess candidate technologies, it is useful to decompose the bio-detection process into four steps:

1. Cueing – Is there a suspicious aerosol cloud present (within range of the sensor)?
2. Detection – Is a biological substance present in the aerosol cloud?
3. Discrimination – Is a biological agent present?
4. Identification – What is the biological agent?

The cueing function leads to triggering subsequent steps in the process. It would be uneconomical to continuously operate sophisticated detection sensors due to the high consumables cost. Therefore, trigger-type sensors are used to cue or initiate subsequent processes to collect, detect and identify suspicious biological material.

Standoff detection technologies are not a subject of this study. These systems were discussed in general in Section 3 in order to present as complete a picture as possible of all of the factors involved in a militarily useful end system. A complete system would employ technologies that would perform all of these functions.

Significant factors in selecting a particular technology or sets of technologies for application to military bio-detection systems are the logistical support and ease of use. Bio-detection technologies can be generally characterized as either “wet” technologies or “dry” technologies. Wet technologies, by their very nature, imply a greater logistical burden and greater difficulty in routine use than do dry technologies. Storage and handling of wet media and reagents are likely to present problems for field users. From a logistical perspective, dry technologies are clearly preferable.

### *4.1 Collection and Sampling Technologies*

The term “sampling” is usually taken to mean the collection of a large volume of air and concentrating the particulate matter in either an air or fluid medium so as to prepare a “sample” for further investigation and analysis. There are but a few technology alternatives in this area. The sampler must have a relatively high efficiency, or ability to collect the appropriate sized particles from a given volume of air. Given that most current collectors are relatively inefficient, they must process a large volume of air as to capture a significant quantity of the biological material for processing and analysis. This usually implies a relatively large air-handling device with associated pumps, fans, and motors. Hundreds of liters of air are normally required to obtain an adequate sample of material, and the collection device must be able to gather that much air and aerosol. The technological advantages to be made in terms of a deployable system are substantial when one considers the potential reduction in size, weight, noise, and power requirements of the sampler component of an integrated bio-detection system.

Several types of samplers/collectors have been evaluated for biological agent detection. The principal differences between collection for biological detection and other types of aerosol or particulate sampling are:

- Biological sampling is normally targeted at living organisms, so the sampling technique must preserve and not harm or alter the collected sample,
- Most biological detection and identification technologies require a liquid sample, so the collection must be from an aerosol or particulate onto a liquid, and
- Since response time is a critical factor, the liquid sample must be highly concentrated and quickly available for analysis.

### 4.1.1 Cyclone Collectors/Samplers

A cyclone collector is an inertial device that is commonly used in industrial applications for removing particles from large airflows. They are attractive because they are inexpensive and do not involve a filter that would require constant maintenance. They appear as a cylindrical body with a conical base and covered top, usually elevated so that a collection hopper can be placed beneath the conical base.

A blower system forces particle-laden air into a tangential inlet at the top. The particle laden air stream forms an outer spiral moving downward towards the bottom of the open-based cone. Larger particles (in our interest, the larger biological particles) are collected on the outer wall due to centrifugal force, are slowed due to aerodynamic effects (skin friction and boundary layer), and fall out the bottom outlet into a hopper or other container. Smaller particles remain in the air stream that forms the inner spiral and follows a path of least resistance, leaving through the upper air outlet in the center of the top. Application of a water spray to the outer walls of a cyclone facilitates particle collection and preservation.

Cyclone collectors have been miniaturized to the point of being man-portable for application in CB detection systems. Cyclone collectors are an application of the physics of fluid dynamics, the fluid in this case being air. The challenge in small cyclone collectors (and any other small collector) is to draw a relatively large air flow through the device so as to be able to collect a meaningful sample from a low concentration aerosol.

### 4.1.2 Virtual Impactors

A conventional impactor operates by accelerating particles through a nozzle. The air stream is directed against an impaction plate maintained at a fixed distance from the nozzle. The plate deflects the flow, and the larger particles are unable to follow the fluid (air, in this case) streamlines (due to the large inertia) and thus impact the plate. Smaller particles follow the fluid streamlines and exit the sample.

A virtual impactor is similar to a conventional impactor but uses a different impaction mechanism. A collection probe replaces the flat plat of the conventional impactor. The collection probe is a tube of larger diameter than the acceleration nozzle. The larger particles penetrate the central collection probe due to their greater inertia. The smaller particles tend to follow the diverted “overflow” around the collection probe.

Air flows through the collection probe, and the collected particles are transported to other portions of the collector for additional concentration. The smaller particles are flushed out of the sampler just as in the conventional impactor. By properly controlling the flows in the impactor, it is possible to adjust the cutoff size of the particles collected. Additionally, the portion of the total airflow that passes through the collection probe represents a smaller percentage of the total flow (10% to 30%), so the virtual impactor is also concentrating the particles into the collection probe airflow. By cascading a series of probes, each taking the flow from the preceding probe, particles can be concentrated to many times the original air concentration before collection. The final stage can then impact the particle stream onto a liquid, delivering a highly concentrated sample.

### 4.1.3 Bubbler/Impingers

Most bubblers or impingers operate by drawing aerosols through a current inlet tube and jet. Usually the jet is submerged into the liquid contained in the sampler. As the air passes through the liquid, the aerosol particles are captured by the liquid surface at the base of the jet. In order to collect the smallest particles possible, the jet is typically made with a small critical orifice causing the flow to become sonic. Other designs have a fitted jet so that tiny air bubbles are formed in the liquid as air leaves the jet.

### 4.1.4 Variable Particle-Size Impactors

The variable particle-size impactors usually have multiple stages. Each stage contains a number of precision-drilled orifices that are appropriate for the size of the particles to be collected in that stage, and orifice sizes decrease with each succeeding impactor state. Particles in the air enter the instrument and are directed towards the collection surface by the jet orifices. Any particle not collected by that stage follows the stream of air around the edge of the collection surface to the next stage. The collection plate is typically a petri dish with agar or other suitable growth

medium. As such, these are applicable to laboratory equipment but not necessarily to field equipment suitable for military application.

### *4.2 Triggering and Detecting Technologies*

Operational and logistic considerations generally limit the extent to which complex sampling, concentration, and identification processes can be accomplished. The approach has been to use a "trigger" to monitor for the presence of suspicious particles and agents. There are currently two general types of triggers – a simple trigger and a more complex trigger. A simple trigger responds to an increase in the atmospheric particulant background count or concentration. Once the threshold is breached, it activates the more complex and precise detector element to determine the nature of the suspected agent. A smart trigger provides the operator with additional information. These systems are capable of providing generic discrimination, i.e., biological vs. non-biological. This trigger may fill the "detect-to-warn" function which allows commanders to order soldiers to don protective gear, adopt a higher protection level or avoid suspected contamination areas. As many detection technologies involve a substantial logistical burden, both in materiel and manpower, the trigger concept is desirable from a systems point of view.

#### *4.2.1 Fluorescence Particle Sizing (FPS)*

Fluorescence Particle Sizing is a combination of aerodynamic particle sizing technology and fluorescence technology in a single system. Aerodynamic particle sizing is described first and the additional fluorescence detection feature follows.

Aerodynamic Particle Sizing (APS) is an example of a simple trigger and is a means for counting the relative number of particles in specific size ranges, useful for non-specific detection as in a trigger element. With APS, the particle laden air stream is drawn into the system through a flow nozzle, producing a controlled high-speed aerosol jet. The air velocity at any point in the flow field stays constant during the measurement period. Individual particles accelerate at different rates within the jet, based on the size of the particles. Thus, particle velocity at any given point is inversely related to the aerodynamic size characteristics of the particle (smaller particles accelerate more rapidly than larger particles). A laser beam is then used to measure the time of flight of the individual particles. The beam is split into two parallel beams, and as the particles pass through them, a pair of electrical pulses is produced by forward-scattered light, collected and sensed with a photomultiplier tube. A high-speed clock measures the time between the electrical pulses (time of flight). The aerodynamic particle size is calculated with a previously stored calibration curve. Particles are thus counted and sized for a specified sampling period, and results displayed as a histogram of aerodynamic diameter versus number.

FPS is an example of smart trigger. It is an APS modified to include an additional laser (blue or ultraviolet) to detect aerosol particle fluorescence in addition to aerodynamic particle size sensing. The second laser beam is located downstream and perpendicular to the standard dual laser beams. In addition to obtaining the aerodynamic particle size, the dual beam laser's signal acts as a trigger to open a time window in which to look for particle fluorescence. A post-processing scheme is used to subtract out background fluorescence signals.

Fluorescence Particle Sizers examine a concentrated aerosol sample for biological fluorescence and compare this response to background particle size characteristics. When compared to background aerosol concentration and particle size distribution from the APS, biological fluorescence has been shown to be a reliable indicator of potentially hazardous biological particles.

The major difference between typical APS and FPS is the ability to discriminate between non-biological and biological aerosols.

#### *4.2.2 Pyrolysis-Gas Chromatography-Ion Mobility Spectrometry (IMS)*

IMS has the potential to be used as a biological trigger if paired with a pyrolysis and gas chromatography system in line before the IMS.

Pyrolysis involves the decomposition of complex organic compounds into chemical signatures through controlled heating to very high temperatures. Gas chromatography is used to separate the components of the resulting mixture

in time. The ion mobility spectrometer measures how rapidly gas-phase ions move through a buffer gas under the influence of an electric field.

A typical IMS comprises an ion-molecule reaction chamber, an ionization source, an ion drift tube, a shutter to allow ions into the drift tube and a Faraday plate to collect ions at the end of the drift tube. A carrier gas, normally air or nitrogen at atmospheric pressure, transports gases or vapors from the material to be analysed into the ionization chamber of the ion mobility spectrometer. The carrier gas and analyte molecules are ionized by an ionization source such as beta radiation, lasers, discharge lamps and partial or corona discharges.[4] Mobility measurements are performed in the drift tube, which contains the buffer gas and usually has a series of electrodes to provide a uniform electric field. The electric field accelerates the ions, while collisions with the buffer gas decelerate them, leading to a constant drift velocity for each type of ion. The mobility is the ratio of the drift velocity to the electric field and it contains information about the interaction between the ion and the buffer gas. For a large polyatomic ion, the mobility depends on the average collision cross-section. An ion with a large average cross-section undergoes more collisions with the buffer gas and travels more slowly than an ion with a small average collision cross-section. Mobility measurements can be used to separate ions with different geometries and several groups have used these measurements to characterize the size distribution of aerosol particles.[5] IMS works in a similar way as a Time-Of-Flight (TOF) mass spectrometer except that the major difference is that a TOF mass spectrometer requires a vacuum where the mean free path length of the gaseous molecules is many times the dimension of the instrument.[6] A mass spectrometer discriminates between the mass to charge ratios of the ions where an IMS discriminates between the mobility (drift velocity vs. electric field) of the ions.

The combination of pyrolysis, gas chromatography, and ion mobility spectrometry is an ingenious combination. Bacterial spores, which are at the low end of the bacterial size range, are too large to be handled by the typical mass spectrometer. Pyrolysis, as an upstream process, reduces the size of the particle and obtains useful information prior to the IMS stage.

### *4.2.3 Flame Photometry and Gas Chromatography (GC)*

Flame photometry, more properly called flame atomic emission spectrometry, is a fast, simple, and sensitive analytical method for the determination of trace metal ions in solution. Because of the very narrow and characteristic emission lines from the gas-phase atoms in the flame plasma, the method is relatively free of interference from other elements. Typical precision and accuracy for analysis of dilute aqueous solutions are about ±1-5% relative.

The method is suitable for many metallic elements, especially for those metals which are easily excited to higher energy levels at flame temperature – sodium, potassium, calcium, rubidium, cesium, copper, and barium. Non-metals generally do not produce isolated neutral atoms in a flame, thus they are not suitable for determination by flame emission spectroscopy.

Flame photometry is an empirical method of analysis - that is, you must calibrate the method carefully. Many different experimental variables affect the intensity of light emitted from the flame. Therefore, careful and frequent calibration is necessary for good results.

Flame photometry and GC are closely related. Flame photometry is a standard laboratory technique for the identification of chemicals. Its use as a biological detection technology is based on the phosphorous content of biological material that is visible to flame photometry sensors. GC takes the process a step further by employing a more sophisticated spectral analysis of the products of combustion.

GC - specifically gas-liquid chromatography - involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase that is adsorbed onto the surface of an inert solid. The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector that is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

The principle of GC is that a gas passed over a solid or liquid surface to which it has some tendency to bind will be "slowed" compared to a gas that passes over the same surface, but has no tendency to bind. The time that it takes for a gas to pass through the column is called its retention time; gases which tend to bind to the column have longer retention times than do gases which do not.[7]

A typical GC column consists of a glass column packed with beads composed of silica gel, activated charcoal, or molecular sieve particles. Longer columns tend to have a higher resolution, with better separation of gases.

The elution characteristics of a gas through a column of this type are quite complex and are affected by the flow of the inert gas, the chemical characteristics of the organic compound, the changing temperature, and the characteristics of the surface to which the gases bind. In practice, this is not a problem, because the elution characteristics of a particular type of column are determined and validated by the manufacturer, who tests a wide variety of substances and then empirically determines their retention times by the column. The retention times are tabulated and are available on-board the computer which controls the GC instrument.

The GC provides considerable information about the identification of a compound, since only a small number of compounds will have retention times of a particular value. Tabulated lists of retention times for thousands of compounds are available both in book form and in computer libraries. However, the GC cannot completely characterize a compound, since more than one substance may have the same retention time.[8]

There are many detectors that can be used in GC. Different detectors will give different types of selectivity. A non-selective detector responds to all compounds except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound. Detectors also can be grouped into concentration dependant detectors and mass-flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample. Dilution with make-up gas will lower the detector’s response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas.

There are several types of detectors used in GC systems. These include flame ionization, thermal conductivity, electron capture, nitrogen-phosphorous, flame photometric, photo ionization, and electrolytic conductivity.

### *4.2.4 Size and Shape Analysis*

The term "particle size" is not always well defined, and it is important to understand just what aspect of particle size a particle instrument actually measures. Instruments such as cascade impactors and time of flight laser spectrometers measure a size that depends on the inertial behavior of the particles and is usually referred to as Aerodynamic Diameter. This is defined as the diameter of a unit density sphere that has the same settling velocity as the particle in question. Instruments which rely purely on light scattering measure an equivalent Optical Diameter which is usually the diameter of a polystyrene latex sphere which produces the same intensity of scattered light on the instrument's detector as does the particle in question. The phase Doppler technique on the other hand measures the radius of curvature of particles and is therefore only suitable for the measurement of particles that have a unique radius of curvature, i.e. spheres. Instruments that rely on optical techniques can be further sub-divided into those instruments that require taking a sample flow of aerosol and those which simply require optical access to the aerosol and are therefore non-invasive. Particles that are smaller than the wavelength of light scatter very little light and are therefore difficult to detect by optical methods. Moreover, the concept of aerodynamic diameter ceases to be relevant for such small particles.[9]

The most common method of characterizing airborne particles is by measurement of the aerosol size distribution. However, many of the techniques used for aerosol sizing are also sensitive to particle shape and can produce misleading results if shape effects are ignored.[10] Moreover, the shape of a suspected biological agent can assist in identifying that agent, when combined with other information.

Size and shape analysis systems have been built to detect biological aerosols. Generally, when a

laser illuminates a stream of particles, the particles produce both forward and back-scattered radiation. Either the forward or back-scattered radiation can be collected on multi-element intensified solid-state arrays then analyzed. The asymmetry of the scattered radiation can be used to determine the size and general shape of the particle (spherical, cylindrical, etc). These systems usually also excite any intrinsic fluorescence in the particles to assist in the discrimination between biological and non-biological particles. Existing systems can analyze from 5000 to 10000 particles per second and measure particle asymmetry reliably to at least one micron.

### *4.2.5 Flow Cytometry*

Cytometry refers to the measurement of both the physical and chemical characteristics of cells. Flow cytometry refers to this same technique in which the characteristic measurements are made as the cells or other particles, which are present in a moving fluid stream, pass through an interrogation point. Modern flow cytometry is a hybrid technology that combines developments in computer processing, opto-electronics, monoclonal antibody production, fluorochrome chemistry, and laser technology to provide an automated method for bio-chemical analysis. The technique permits characterization and identification of biochemical species (cells, viruses or toxins) within a heterogeneous mixture of organic and inorganic material. Over the past 30 years, flow cytometry has evolved from a few custom-made devices to many commercially available instruments used routinely in many areas of biomedical research and clinical diagnostics.

Flow cytometry is a means of measuring certain physical and chemical characteristics of cells or particles as they travel in suspension one-by-one past a sensing point. In one way, flow cytometers can be considered to be specialized fluorescence microscopes. The modern flow cytometer consists of a light source, collection optics, electronics and a computer to translate signals to data. In most modern cytometers the light source of choice is a laser which emits coherent light at a specified wavelength. Two lenses (one set in front of the light source and one set at right angles) collect scattered and emitted fluorescent light and by a series of optics, beam splitters and filters, specific bands of fluorescence can be measured. We can measure physical characteristics, such as cell size, shape and internal complexity and, of course, any cell component or function that can be detected by a fluorescent compound can be examined. The applications of flow cytometry are numerous, and this has led to the widespread use of these instruments in the biological and medical fields.[11]

The following table lists the structural characteristics of biological cells that are measurable using flow cytometry technology.

**Table 4.1, Structural Characteristics of Biological Cells Measurable by Flow Cytometry**

| Parameter | Measurement Method |
|---|---|
| Cell size | Extinction or small angle light scattering |
| Cell shape | Pulse shape analysis |
| Cytoplasmic granularity | Large angle light scattering, electronic impedance |
| Birefringence | Polarized light scattering |

A flow cytometer is a particle counting and sizing device that uses a liquid sample in which aerosol particles have been separated into their sub-components. The sample to be analyzed is injected into the center of a fast moving fluid stream that is then forced through an opening. Upon exiting the opening, the stream passes through a measurement station where particulates, such as bacterial cells, are illuminated by a light source. Scattered light is measured at different angles and wavelengths using a series of optical filters and photomultiplier tubes to provide information concerning the cell's size, shape, and fluorescence. A specially formulated dye is added to liquid samples and then placed in the flow cytometer. The instrument automatically processes the samples and displays the results to the operator. Pattern recognition techniques, based on particle size and fluorescence, are used to differentiate bacteria from natural airborne biological materials such as pollens and mold spores.

The advantages of flow cytometry for bio-sensing include: fast sample preparation and analysis, single particle analysis, detection and identification in one instrument, significant multiplexing advantages, easily quantifiable results, adaptability to high, automated throughput, simple to operate, and compact instrumentation.

The limitations of flow cytometry for bio-sensing include: the lack of trigger/detect algorithms that fully utilize the capability of the technique, that most instruments to date have been designed for the clinical laboratory and not the field and there tends to be a high logistics burden to support flow cytometry instruments.

### *4.3 Identification Technologies*

Identification technologies come into play when it is necessary to determine specific biological agents in order to determine subsequent treatment of exposed personnel (or "detect to treat"). Detection technologies are generally employed to provide some advance warning of a biological attack such that protective measures can be taken. As such, identification technologies tend to be derived from laboratory processes and equipment. Although many groupings of technologies are possible, this section groups identification technologies under the generic taxonomy of mass spectrometry, antibody based identification, DNA based identification and Raman scattering.

#### *4.3.1 Mass Spectrometry (MS)*

Mass spectrometers use the difference in mass-to-charge ratio of ionized atoms or molecules to separate them from each other. MS is therefore useful for quantification of atoms or molecules and also for determining chemical and structural information about molecules. Molecules, including biological molecules, have distinctive fragmentation patterns that provide structural information that can be used to identify structural components. The analysis of mass spectroscopy information involves the re-assembling of fragments, working backwards to generate the original molecule.

The general operation of a mass spectrometer is to create gas-phase ions, to separate the ions in space or time based on their mass-to-charge ratio, and to measure the quantity of ions of each mass-to-charge ratio. The power of a mass spectrometer to separate ions based on their mass-to-charge ratio is described as its resolution. Resolution is defined as $R = m/m$ where m is the ion mass and $m$ is the difference in mass between two resolvable peaks in a mass spectrum. For example, a mass spectrometer with a resolution of 1000 can resolve between two ions, one with a mass-to-charge ratio of 100.0 and the other with a mass-to-charge ratio of 100.1. A mass spectrometer generally consists of an ion source to create gas-phase ions, a mass-selective analyzer to separate the ions in space or time based on their mass-to-charge ratios, and an ion detector to measure the quantity of ions of each mass-to-charge ratio. Mass spectrometers operate in a high-vacuum environment.

Mass spectrometers utilize a variety of ionization techniques. Different techniques produce varying degrees of fragmentation of organic compounds and therefore produce varying results for analysis.

- Electron Impact Ionization is one of the most commonly used methods of ionization in organic mass spectrometry. Ions are formed by bombarding gas phase sample molecules with a 70 electron-volt beam of electrons. A significant population of the resulting ions will have large excess energy and may decompose to form fragment ions. The resulting mass spectrum can not only

yield useful molecular weight information but the fragment ions also can provide a useful aid in determining the structure of the molecule.[12]

- Chemical Ionization uses a reagent ion to transfer protons to (or from) the sample resulting in the formation of pseudo-molecular ions. The resulting ions tend to fragment much less than with electron impact ionization.[13]
- Fast Atom Bombardment (FAB) Ionization works best for polar and higher molecular weight compounds such as peptides and other biomolecules. Prior to the advent of electrospray ionization and Matrix Assisted Laser Desorption and Ionization (MALDI), FAB provided mass spectra of samples where other methods failed. FAB utilizes a fast moving beam of neutral atoms which bombard a metal target coated with a liquid matrix in which the sample has been dissolved.[14] Typically pseudo-molecular ions are formed, together with fragment ions at lower mass.
- Electrospray Ionization (ESI) is one of the more recent ionization techniques. In ESI, a dilute solution of the analyte is slowly pumped through a short piece of capillary tubing. The capillary is held at several kilovolts with respect to the counter electrode around a centimeter away. The strong electric field at the end of the capillary pulls the solution into a Taylor cone, and at the tip of the cone the solution is nebulized into small charged droplets. As the charged droplets travel towards the counter electrode, they evaporate solvent to ultimately yield molecular ions. The ions are sucked into the vacuum chamber through a small aperture or another piece of capillary tubing (which is usually heated to ensure that the ions are completely desolvated). ESI is a very gentle ionization technique that can leave unsolvated protein ions with a memory of their solution phase structure. Weakly bound complexes (such as those between an enzyme and its substrate) also can be studied by ESI. ESI is a very sensitive technique, which is ideally suited for the analysis of small amounts of large and/or labile molecules such as peptides, proteins, organometallics, and polymers.[15]
- In MALDI the analyte is diluted (usually at around one part in 100-50,000) in a solid or liquid matrix which strongly adsorbs laser light. The matrix is usually a large organic acid, like 2,5-hydroxybenzoic acid. When irradiated with a pulsed laser, the matrix adsorbs light. This radiation results in a sudden local temperature rise which causes the matrix to literally explode into vacuum carrying along the analyte. Proton transfer from photo-excited matrix molecules ionizes the analyte. Since the signal from MALDI is pulsed, this ionization method is ideally suited to time of flight MS.

Mass spectrometers utilize a calibrated analyzer to quantify the ions by their mass-to-charge ratios. There are several types of mass spectrometer analyzers.

- Fourier-transform mass spectrometers take advantage of ion-cyclotron resonance to select and detect ions.
- Ion-trap mass spectrometers use three electrodes to trap ions in a small volume. The mass analyzer consists of a ring electrode separating two hemispherical electrodes. A mass spectrum is obtained by changing the electrode voltages to eject the ions from the ion trap.
- A time-of-flight mass spectrometer uses the differences in transit time through a drift region to separate ions of different masses. It operates in a pulsed mode so ions must be produced or extracted in pulses. An electric field accelerates all ions into a field-free drift region.[16] Fragments drift through a vacuum, with drift speed depending on fragment mass. Lighter ions have a higher velocity than heavier ions and reach the detector at the end of the drift region sooner.
- A quadrupole mass filter consists of four parallel metal rods. Two opposite rods have an applied potential opposite to the two other rods. The applied voltages affect the trajectory of ions traveling down the flight path centered between the four rods. For given direct current and alternating current voltages, only ions of a certain mass-to-charge ratio pass through the quadrupole filter, and all other ions are thrown out of their original path. A mass spectrum is obtained by monitoring the ions passing through the quadrupole filter as the voltages on the rods are varied. There are two methods: varying the frequency while holding the voltages constant, or varying the voltages while holding the frequency constant.

#### 4.3.1.1 Tandem Mass Spectrometry (MS/MS)

MS/MS employs two mass spectrometers in tandem. Between the two analyzers is a collision gas cell. Precursor ions selected by the first MS collide with a high pressure gas (usually helium) in the cell and undergo fragmentation. The second MS analyzes the resulting daughter ions. The collision process is called Collision Induced Dissociation (CID). MS/MS is used for the structural studies of complex molecules. Many large molecules such as peptides have spectra with only a few fragment ions. MS/MS has proven useful in the sequence determination of peptides due to the formation of abundant daughter ions in the CID process.[17]

### 4.3.2 Antibody-Based Identification

The immune system is a well-designed fortress that defends its host against foreign invasion. The sentinels of this fortress are macrophages that continually roam the bloodstream of their host. When challenged by infection or immunization, macrophages respond by engulfing invaders marked with foreign molecules (antigens). This event, mediated by helper T-cells, sets forth a complicated chain of responses that result in the stimulation of B-cells. These B-cells, in turn, produce proteins called antibodies that bind to the foreign invader. The binding event between antibody and antigen marks the foreign invader for destruction via phagocytosis or activation of the complement system.[18]

Five different classes of antibodies (or Immunoglobulins (Ig)) exist: IgA, IgD, IgE, IgG, IgM. From a structural point of view, IgG antibodies are a particular class of immunoglobulins that have been extensively studied, perhaps because of the dominant role they play in a mature immune response. IgG antibodies are Y-shaped proteins composed of two heavy chains and two light chains that are joined by disulphide linkages. The IgG molecule can be broken down into two regions, the Fc and Fab. The Fc region, so called because it is the fragment of the IgG molecule that most readily crystallizes, is involved in effecting the physiological roles the antibody must play. Two identical Fab fragments are present at the ends of the "Y" in every IgG structure. The Fab region is named as such because it is the IgG fragment that contains the antibody-binding site. The Fab region contains a region of highly conserved amino acids as well as a region of highly variable amino acids. These variable sequences are confined to six protein loops (or complementarity determining regions) that cluster together at the end of the Fab fragments to form a continuous hypervariable surface. It is this region that is responsible for the binding of foreign antigens.

In order to perform their crucial role in the line of defense, antibodies must be extremely versatile. Indeed, on even a daily basis the immune system encounters a great variety of foreign substances (e.g. bacteria, viruses, toxins). As a result, antibodies must be extremely diverse to counter a large number of unexpected and unknown possibilities.[19]

Another needed attribute of antibodies is specificity. In order to distinguish between both self and a multitude of foreign species, antibodies need to have a highly discriminating method of recognition on the molecular level. This specificity is the result of the complementary nature of antibody binding.[20] This characteristic of antibody binding is the result of immunologically-tuned interactions (i.e. charge-charge, dipole-dipole, H-bonding, and Van der Waals) between the antigen and amino acid residues present in the antibody binding pocket. By taking advantage of the varied chemical properties of the 20 amino acids, the immune system is able to generate an array of antibody binding pockets that can accommodate the shape, charge, and hydrophobicity of seemingly any given antigen.[21] The high degree of complementarity exhibited by antibody binding also endows antibodies with high affinities for their antigens.

#### 4.3.2.1 Production and Isolation of Polyclonal and Monoclonal Antibodies

Two types of antibody samples can be used in the study of antibody-related phenomenon. The first type, polyclonal antibodies, can be obtained by immunizing a mammal, such as a goat, sheep, mouse, or, most conveniently, a rabbit. After immunization, blood is removed (periodically, if desired) and the antibodies can be purified directly from the serum. The name polyclonal is derived from the Greek word for many (polys) and sprout (klon). As implied by the name, polyclonal antibodies originate (or "sprout") from a variety of B-cells that differ in the genetic

material that encodes for antibody production. In a polyclonal sample, some of the antibodies will be specific for the antigen with which the animal was immunized. The remaining antibodies have been elicited from encounters with other foreign antigens that the animal has been exposed to throughout its lifetime.

The second type of antibody sample, the monoclonal antibody, is derived from a more complex process. A mammal, almost always an inbred mouse, is immunized with an antigen. After repeated immunizations, the spleen of the animal is removed. Because the spleen is responsible for B-cell production, the spleen cells contain the genetic information that gives rise to antibody production. Unfortunately, these spleen cells cannot be cultured. As a result, they are fused with "immortal" myeloma cells, so-called because of their ability to proliferate in vitro. The resulting fused cells, called hybridoma cells, are screened with a colorometric enzyme-linked immunoabsorbant assay (ELISA). Use of this assay allows for the selection of hybridoma cells that produce antigen-specific antibodies. Because a given hybridoma cell is derived from a single B-cell, it produces a monoclonal antibody. Here, the prefix mono-, derived from the Greek word for single (monos), is used to indicate that a monoclonal antibody is derived from the genetic code of a unique B-cell. Once a single hybridoma line is selected, it is injected into a healthy mouse. Hybridoma cells, like myeloma cells, have the ability to produce tumors; consequently, after injection with a hybridoma line, a tumor grows inside the host mouse. When this tumor grows, it produces ascites, a fluid that is rich in monoclonal antibodies. Once antibodies are produced, considerable care needs to be taken in their purification to avoid deleterious effects that may affect their study.

Depending on the experimental situation, either a polyclonal or monoclonal antibody approach may be warranted. Each approach offers certain advantages. In the case of polyclonal antibodies, there are clear technical advantages. Polyclonal antibodies are inexpensive to produce relative to the cost of monoclonal antibody technology. In addition, large quantities of polyclonal antibodies (~10 mg/mL) can be produced from the serum of an immunized animal. Finally, high affinity polyclonal antibodies can be isolated merely 2-3 months after the initial immunization. This expeditious production facilitates their rapid study. There also are advantages to the use of polyclonal antibodies from a scientific perspective. Because polyclonal antibodies contain the entire antigen-specific antibody population, they offer a statistically relevant glimpse into the overall picture of an immune response. A similar viewpoint is considerably more difficult, if not impossible, using a monoclonal antibody approach.

On the other hand, monoclonal antibodies have certain advantages over polyclonal antibodies. Because of their immortal nature, hybridoma cells can be frozen, thawed, and recultured in vitro. As a result, for a given monoclonal line, there exists a constant and renewable source of antibodies for study. In addition, the defined composition of a monoclonal antibody allows for its chemical composition, on a molecular level, to be analyzed in detail. For example, X-ray crystallographic and gene sequencing methods can only be applied to monoclonal antibodies. This level of detail is particularly useful when studying mechanistic issues related to binding.[22]

#### *4.3.2.2 Antibody-Based Sensors*

Many different types of antibody-based sensors have been developed. They include the fiber-optic biosensor and the continuous flow immuno-sensor for on-site screening and monitoring of contaminants. Both sensors determine the level of contamination by measuring the level of fluorescent activity caused by the introduction of a biological sample to the system. The fiber-optic biosensor works when contaminant molecules compete with fluorescent antibodies on the sensor. A decrease in fluorescent activity caused by contaminants binding onto antibody sites corresponds to the level of biological agent present. The continuous flow immunosensor works when the agent molecules displace fluorescent antibodies that are placed on a solid support. These displaced antibodies are then detected and correspond proportionally to the level of concentration.

Antibody-based probes (immuno-sensors) offer a highly specific probe technology, since antibodies recognize very specific sites or cellular components (epitopes). Antibodies specific for any microbe can be made if the microbe can be obtained in pure culture. These must be screened for binding characteristics, that is, binding affinity, on- and off-rates, and epitope recognized. The production of monoclonal

antibodies requires significantly more time and effort in the development of hybridoma cell lines with appropriate characteristics. It is, therefore, desirable to provide for breaking the antibody-antigen bond after a positive test and reusing the antibody in additional tests. The binding of the target (antigen) to the antibody can be monitored directly with a transduction method, such as luminescence or electrochemical signal. Alternatively, the binding can be monitored in a sandwich assay in which a second antibody labeled with a fluorescent dye binds to another epitope on the captured cell or to the probe antibody. Indirect methods monitor the bound epitope by its competition with a standard epitope labeled with a fluorescent dye. While this indirect format is more sensitive, the antibody must bind very strongly to the antigen target.

Fluorescence-based fiber optic immunosensors have demonstrated the detection of $10^4$microbial cells/ml, and immuno-electrochemical sensors have demonstrated $10^3$ cells/ml. Problems include nonspecific binding, degradation of the antibodies over time, reproducibility of the antibodies, and whether the target can be produced in pure culture to provide a monoclonal antibody. There also is a problem with cross-reactivity, that is, closely related organisms frequently cannot be distinguished by immuno-chemical techniques. In addition, some viruses possess hyper-variable coat proteins, and a monoclonal antibody raised against a particular coat protein of a virus may be totally useless for detection of the same virus after it has been propagated for several generations.[23]

#### *4.3.2.3 Capillary Electrophoresis (CE)*

CE involves the application of a high electrical potential across two vessels containing a substance of interest in a buffer solution. The electrical potential causes capillary flow, and a sensor in the capillary provides the output.

Electrophoresis refers to the migration of charged electrical species when dissolved, or suspended, in an electrolyte through which an electric current is passed. Cations migrate toward the negatively charged electrode and anions are attracted toward the positively charged electrode. Neutral solutes are not attracted to either electrode. Conventionally, electrophoresis has been performed on layers of gel or paper. The traditional electrophoresis equipment offered a low level of automation and long analysis times. Detection of the separated bands was performed by post-separation visualisation. The analysis times were long as only relatively low voltages could be applied before excessive heat formation caused loss of separation.[24]

Performing electrophoretic separations in capillaries offers the possibility of automated analytical equipment, fast analysis times and on-line detection of the separated peaks. Heat generated inside the capillary is effectively dissipated through the walls of the capillary which allows high voltages to be used to achieve rapid separations. The capillary passes through a detector, usually an ultra-violet (UV) absorbance detector. The majority of instruments also have UV diode detectors available. Alternative detector modes commercially available include fluorescence, laser induced fluorescence, conductivity and indirect detection. A resulting plot of detector response with time is generated and is termed an electropherogram.

The capillary also can be filled with a gel, which eliminates the electro-osmotic flow. Separation is accomplished as in conventional gel electrophoresis but the capillary allows higher resolution, greater sensitivity, and on-line detection

#### *4.3.2.4 Ion Channel Switch (ICS)*

Channels are integral membrane proteins that enable rapid yet selective flux of ions across biological membranes. They are central to the electrical properties of excitable cells (e.g. neurons). However, they are found in membranes from a wide range of organisms, including viruses, bacteria and plants. The Australian Membrane and Biotechnology Research Institute (AMBRI) has built a biological switch, a membrane which can detect the presence of specific particles, and signal their presence by triggering an electrical current. This device - the ICS Biosensor - is based on and made possible by two novel nanoscale building blocks:

1. A "sliding switch", using two halves of a molecule which behaves like a channel - a tube which will let charged particles flow through it. When the two halves line up, the switch is "on", and the ions flow. When the two halves are separated (by sliding

sideways), the ion flow is cut, and the switch is "off". The two halves of the molecule move in the upper and lower layers of a membrane.

2. An electrode which has a miniature reservoir formed between its surface and a biological membrane made from two layers of lipids. The "bottom" layer of lipid is attached to the electrode by linking molecules.

The AMBRI sensor consists of a two layer self-assembled membrane. The bottom layer is attached to a gold film deposited on glass, the top layer is free to move around. Both layers include gramicidin molecules, which act as ion channels through the membranes. The top layer is covered with the solution to include the molecule to be detected. Antibody fragments which have affinity only for the molecule to be detected are tethered either directly to the second layer of the membrane or to a gramicidin molecule in the second layer, via a streptavidin molecule and a biotin linker.

A voltage is imposed between a platinum wire immersed in the solution and the gold film, but the membrane prevents current (ions) from flowing unless the gramacidin molecules form transient ion channels as the second layer moves randomly over the first. Due to the large number of gramacidin molecules included in both layers, a steady flow of ions pass, causing a measurable current. The gauge shows the current flowing.

When the molecule to be detected is introduced into the solution above the layers, they will bind to the antibodies, which prevents the gramacidin molecules in the top layer from moving for some time. This prevents ions from flowing through the layers, which causes a drop in the measured current. The rate of current drop is proportional to the concentration of detected molecules present.[25] The features of this system include that it functions at a nanoscale level – a level of single molecules. A single channel can allow a flow of up to a million ions a second. It is not easily fouled by proteins and other components of blood or serum, and it can be reduced in scale to sizes comparable to and smaller than existing microelectronics. Researchers anticipate that they can achieve higher resolution and increased sensitivity due to the fact that this device can be scaled to very small dimensions. As the membrane area is reduced, any leakage decreases proportionately, yet the conductance per channel remains constant. This means that with small electrodes (<30µm diameter), it becomes possible to resolve current transients of individual channels. Multi electrode arrays of such electrodes could further increase the sensitivity of the device.

AMBRI researchers pointed out that the ICS Biosensor does not use life itself. Instead, it lays an artificial cell membrane atop a gold electrode. The membrane functions like a wall with many gates. Each gate is controlled by a molecule that closes the passage when it runs into a particular target molecule. When it is present, the gates swing shut, electrical flow from one side of the membrane to the other slows, and the electrode registers a change in impedance. The change is proportional to the amount of drug in the sample. AMBRI scientists maintain that this design makes it easy to create a wide variety of very sensitive and stable biosensors by simple switching of the doorman molecules. So far they have built chips that can detect viruses, bacteria, drugs, proteins, DNA sequences, and minerals such as potassium and calcium. Their tests show that the sensors can accurately measure levels of target compounds present in blood, serum and urine samples. These biosensors appear to remain stable over a wide range of temperatures.

#### *4.3.2.5 Tissue-Based Bio-sensors*

Research is being conducted into the potential of developing innovative cell and multi-cellular tissue-based sensors. With the increasing ability to modify and engineer potential agents, the ability to detect agents that have not been identified or fingerprinted at the molecular level has become more important. Multi-cellular assemblies and the communications between cells in a tissue environment may be useful components for devices that are responsive to a wide range of agents and provide a more predictable assessment of the physiological consequences of exposure than specific antibody or other sensors.

The wide variety of cells and tissues that the human body uses for detection and defense of toxins could be used as physiological based biosensors that functionally respond to known and unknown biological, chemical, or physical stimuli. Cells that could form the basis of the tissue-based biosensor may be primary or transformed cells from a variety of sources including neurons, immune cells, endothelial

cells, fibroblasts, myocytes, primordial and peripheral stem cells, etc.

Enhanced performance could be demonstrated by examination of patterned or random co-cultures of cells with accompanying support cells in tissue-like environments or organotypic cultures in adherent or flow-through systems. The construction of three-dimensional scaffolds or materials which support the fabrication of multi-cellular arrays or tissues able to respond and report on a wider spectrum of stimuli (e.g., neurotoxins and inflammatory agents) could be examined to allow incorporation of different cell types into the sensor.

Researchers believe that recent advancements in engineering differentiated or undifferentiated primordial cells represent an opportunity to engineer functional responses of cells and their organization into a three dimensional matrix for a tissue based biosensor. Technologies which explore the use of reporter molecules (e.g., fluorescence and luminescence) present an opportunity to report on cellular reactions of importance to functional responses to agents of interest. In addition, the successful development of a tissue based biosensor will require the development of new materials that provide scaffolds for the long-term function of cells in a three dimensional environment.

Issues in the construction of a cell- or tissue-based biosensor include:

- Nutrient requirements
- Efficient fluid transport of nutrients and wastes
- Spatial requirements of cells within the matrix
- Signal processing and information extraction from electrical, optical, mechanical, or other
- outputs from incorporated cells
- Stability and functional turnover of components.

#### *4.3.2.6 Hand Held Immunochromatographic Assays (HHA)*

The HHA is a simple, antibody-based assay used to identify biological warfare agents. HHAs are inexpensive and very reliable. HHAs are designed to identify one agent per assay and can currently identify eight different biological warfare threat and four simulant agents.[26] The Joint Project Manager for Biological Defense manages the Critical Reagents Program which in turn is responsible for the production, quality assurance/quality control, stockpiling and security of reagents as well as the production of HHAs.

To use HHAs, a small quantity of solution containing the suspected agent is placed in a well on the assay. Over a 15-minute period, the solution wicks through the assay where it is successively exposed to different antibodies. The first antibodies flow up the assay as soon as they come in contact with the solution and bind themselves to the specific biological warfare agent, if it is present. A second region of antibodies is moored to the assay's test area, where they immobilize the biological warfare agent (along with the bound antibodies). An enzyme attached to the moored antibody changes the color of a coating on the assay when this antibody binds to the biological warfare agent. A change in the color of this region is evidence of a positive test. Any of the first antibodies that lack the biological warfare agent continue to move into the control region, where they are grabbed by the third type of antibody that is moored to the control region of the assay. Again, an enzyme attached to this moored control antibody changes the color of a coating on the assay when exposed to the first, flowing antibody. Coloring of this second region only indicates that the antibodies are behaving properly but is not an indication of exposure to the biological warfare agent.[27]

Single step HHAs offer the advantages of low cost and simplicity to use but the sensitivity of these devices is much lower than that achieved in clinical laboratories.[28] The HHA has a one-time only use capability and cannot be reused once fluid is applied. They must also be disposed of as medical waste. The user must wait 15 minutes before interpreting the results – shorter exposure times could give false negative results and longer time may give false positives as the labeled antibody can start to flow back down the assay. The colored indications are not permanent and will fade quickly with time. Storage life of HHAs at refrigeration temperatures is over two years, but at room temperature this shortens to eight weeks and is further reduced to two weeks at 45 degrees Celcius. Cycling the HHAs between temperatures will reduce their sensitivities and must be avoided.[29]

To overcome the lack of sensitivity and occasional false positive of traditional HHAs, U.S. Army Solider and Biological Command (SBCCOM) and the U.S. Army Research Laboratories are investigating dendrimer-based tickets. They have developed a nanomanipulation strategy that allows binding receptors (antibodies) to be oriented at a nanoscopic scale through a self-assembly process. So far, a variety of nanostructured polymeric materials have been synthesized and tested. Among them, the rigid, spherical, tree-like dendrimers are the best nanostructured polymers capable of orienting the antibody binding direction at different surfaces. As a result, HHA tickets have been significantly enhanced, and the detection time has been dramatically shortened. Moreover, after the introduction of "cheap" synthetic protein-like dendrimers, the ticket production cost also has been significantly reduced. Dendrimers also are being used in a new fluorescence signal amplification strategy for biological agent detection. While only one flourescein group can be linked onto an antibody molecule, a large number of fluorophores can be attached to an antibody through a dendrimer linker molecule, thus forming a water-soluable fluorescein-dendrimer-antibody bioconjugate. Upon addition of the antigen, enhanced fluorescence signals are obtained with fluorescein-dendrimer-antibody as compared to the corresponding fluorescein-antibody analogs.[30] Dendrimer-based HHAs offer the potential advantages of reduced production cost (by a factor of approximately two), lower false positive indications, extended shelf life stability, significant improvement in lot-to-lot variability and the potential for mass production.

#### *4.3.2.7 SMART® Tickets*

SMART®, in this case, is an acronym for the commercially available Sensitive Membrane Antigen Rapid Test. SMART® is a registered trademark of New Horizons Diagnostics Corporation. The SMART® identification tickets are self-contained, colorimetric, solid-phase immuno-filtration assays designed to be used in conjunction with a liquid interface. Two types of SMART® devices have been developed: One kit is capable of detecting endospore-forming bacteria. The other kit is capable of detecting proteinaceous toxins or soluble antigens, including bacteria. The SMART® devices utilize a colloidal gold particle concentration immunoassay to effect sensitive and selective detection of biological materials. Antibodies specific to the agent of interest are conjugated to colloidal gold particles. When concentrated on solid surfaces, these particles can be seen by the naked eye. Labeled antibodies can easily be lyophilized and reconstituted without losing activity or specificity.

The presence or absence of the target antigen is indicated colorimetrically. A small red dot appears on the ticket that the user compares with a color chart.

SMART® tickets to detect anthrax and botulinum toxin were issued to the military during Operation Desert Storm in 1991. The technology is further developed and incorporated in a variety of the BW detection devices now in the field.

#### *4.3.2.8 Fiber Optic Waveguide*

A fiber optic waveguide uses fiber optic material designed to confine and direct light along its length. Optical fibers are thin strands of super-clean glass (fused silica), about the size of a human hair. The basic design of an optical fiber consists of two components - the core and the cladding. Optical waveguides conduct optical power (photons) in the form of light rays. Core and cladding differ primarily in the refractive index of the glass. The core's refractive index is slightly higher than the cladding's, thereby creating a boundary for a circular waveguide. A fiber optic sensor in general will consist of a source of light, a length of sensing (and transmission) fiber, a photo-detector, demodulator, processing and display optics and the required electronics. These long thin strands of transparent material convey electromagnetic energy in the optical waveform longitudinally by means of internal reflection.

The Naval Research Laboratory (NRL) is researching real-time fluoro-immunoassays for multiple agents using this technology. The fiber-optic evanescent waveguide biodetector developed by the NRL uses antibody probes, some of which are bound to a glass optical fiber immersed in a capillary tube containing an aqueous solution of the sample. Other antibodies, tagged with a fluorescent dye, are added to the sample, where they bind to the target antigen. The antigen-labeled antibody complex then binds to the immobilized antibody. Light from a near infrared diode laser travels

through the fiber, which contains it almost completely. The very small amount of light escaping, the evanescent wave, excites the fluorescent tag, whose emission is sent back up the fiber and detected via a photodiode.[31] This requires adaptation of miniaturized instrumentation and new fluorescent labels. Since the measurement occurs at the fiber's surface in the evanescent wave, users only detect bound fluorophores. NRL researchers believe that this methodology improves the speed, sensitivity, and utility of immunoassays.

This technology has been commercialized under a license to Research International.

#### *4.3.2.9 Surface Plasmon Resonance (SPR)*

SPR is a quantum effect arising from the interaction of light reflecting from a metal surface. Under certain conditions, the energy carried by photons of light is transferred to packets of electrons, called plasmons, on a metal surface. Energy transfer occurs only at a specific resonance wavelength of the incident light, and the resultant effect is absorption of the light at this resonance wavelength.

The SPR resonance wavelength is determined by three factors: the metal, the structure of the metal's surface, and the nature of the medium in contact with the metal's surface. The plasmon's electric field extends about 100 to 200 nanometers in a direction perpendicular to the metal surface. Any change in the material within the plasmon's field causes a change in the resonant wavelength (i.e., resonance wavelength shift). The practical consequence of this extended electric field is that the SPR effect can directly monitor antibody-antigen binding when the antibodies are coated on the metal surface. No tag molecule is required to detect the binding event. Further, the amount of resonant wavelength shift, SPR shift, is proportional to the amount of binding that takes place. A capture antibody is immobilized on the metal surface. The antigen (analyte) binds to the antibody, and this binding event is read by the instrument as a SPR shift.

Advantages include the fact that no reagents are used during a test. This means that consecutive negative samples can flow into the cell between buffer wash cycles without using any reagent or other disposable component. After a positive test, the sensor and/or flow cell must be replaced.

Disadvantages include the fact that only one analyte can be measured at a time and that sensor change-out is currently a manual process.

#### *4.3.2.10 Resonant Mirror*

The resonant mirror biosensor was developed to combine the simple construction of SPR sensors with the sensitivity of waveguide devices. Although SPR sensors and waveguide construction and operation are very different from each other, the resonant mirror balances the properties of both of these sensors into a highly sensitive yet simple device.

The resonant mirror biosensor consists of four layers: the sensing surface, the high refractive index dielectric resonant layer, the low index coupling layer, and a prism. Polarised laser light illuminates the underside of the sensor surface at angles greater than the critical angle. The light is totally internally reflected and illuminates the detector array. A series of polarising filters are incorporated such that any light which follows this path is blocked before reaching the detectors. At one angle, called the resonant angle, a component of the light can couple through the low refractive index spacer layer and propagate along the high refractive index guiding layer. This light can then couple back out, be able to pass through the filter system [due to a polarity change on wave-guide] and appear as a peak of intensity on an otherwise dark background. The angle where this coupling occurs, the resonant angle, is, essentially, dependent upon the refractive index at the surface of the sensor [within the evanescent field]. Hence, changes in refractive index [or mass] will change the resonant angle. So, as mass increases at the binding surface the signal will increase, and as mass decreases at the dissociation surface the signal will decrease. This change in angle is linear with respect to mass.[32]

Maximum sensitivity can be achieved by considering two parameters: the sensitivity of the resonance angle to changes in the sensing layer and the resolution in the resonance angle. The resonance angle sensitivity increases as the amount of the resonant mode within the sensing layer increases. This parameter is controlled by choosing a coupling layer material with as low of a refractive index as possible, shifting the mode

towards the sensing interface. The dielectric layer should have a very high index of refraction and still satisfy the minimum necessary to convey the resonant mode.

The resolution sensitivity is determined by the loss in the system from the material and device sources, or the resonance width. A decrease in resonance width increases the propagation length. Material related losses occur primarily from the scattering of light by irregularities in the film, which changes as the refractive index of the resonant layer changes. Device related losses include the coupling loss, which is reduced by lowering the thickness and refractive index of the coupling layer. However, decreasing the coupling losses will lower the ability to transmit light into and out of the device effectively. A balance of the coupling loss slightly less than the material losses will assure good light transmission and low losses.

The fabrication of the resonant mirror is rather simple. Sputtering and ion beam assisted evaporation techniques are used to make the devices. Because of these methods, large quantities of uniform devices can be made inexpensively.

#### *4.3.2.11 Up-Converting Phosphor Technology*

Optical transduction is employed in many bio-detectors. Although a variety of methods based on light scattering and absorbency have been explored in other settings, many of the optical examples in our inventory involve fluorescence and other luminescence spectroscopies. Fluorescence approaches involve excitation of the molecules of a material with light, usually in the UV portion of the spectrum. The excited component spontaneously reverts to its unexcited state, a process accompanied by emission of light at different wavelengths. These emission wavelengths are dependent upon both the exciting wavelength and the molecules being irradiated, so it is possible to use the resulting emission spectrum to identify the irradiated material. Unfortunately, many biological materials, for example tryptophan, are naturally fluorescent. Due to a number of factors, including the presence of common substances like tryptophan, the luminescence characteristics of many biological and environmental substances overlap-often making identification difficult, if not impossible. However, a variety of methods have been developed to separate individual contributions and the background. An indirect approach involves introducing a special fluorophore (a fluorescing chemical with a distinctive emission spectrum) into the sample or the probe molecule prior to irradiation.

Phosphor particles have been used for decades in television screens and fluorescent tubes. When UV light strikes the phosphor-coated area in a screen or bulb, it excites the particles and colored light is produced. STC Technology has patented improvements on this technology that employ chemical changes within the phosphor particles so that infrared light can be used to produce the colored signal. This use of infrared light rather than ultraviolet light to create a colored signal is called up-conversion versus the down-conversion that occurs with UV light.[33] Since up-conversion does not occur in nature, a detector employing up-conversion technology with biological samples would not experience background interference when excited by infrared light.

Up-converting phosphor technology is being funded through Defense Advanced Research Projects Agency (DARPA) to Stanford Research Institute (SRI). In the SRI design, the phosphor particles are attached to detection probes, antibodies, or DNA that direct the phosphors to bind to antigens such as BW agents. If the target antigen is present, an infrared diode laser causes the phosphor probe to emit visible light. SRI has so far developed nine up-converting phosphors, each producing a different color. This "multiplexing" allows for the simultaneous detection of several agents in the same sample.[34] More phosphors are under development, although it seems likely that the multiplexing limit will probably be closer to nine than to 100.[35] The advantages of up-converting phosphors is:

- single particle detection sensitivity,
- multiplexing,
- no autofluorescence, and
- no photobleaching.[36]

#### *4.3.2.12 Electrochemical Luminescence (ECL)*

ORIGEN is a proprietary detection technology developed and patented by IGEN International, Inc. It is based on ECL, a well-established

process in which certain chemical compounds emit light when electrochemically stimulated. ORIGEN uses these light-emitting compounds as labels for sensing biological compounds in highly sensitive and precise assays.[37]

The heart of IGEN's ECL-analyzer system is an electrochemical flow cell with a photomultiplier tube placed just above the working electrode for efficient light detection. In order to deliver the molecule of interest to the electrode surface, magnetic bead tagging has been implemented into the system's design: Under the working electrode a magnet is positioned for either capture or release of the beads coated with target molecules. Automated sample handling equipment and a fluidics delivery system round out the system.

Although the standard ORIGEN assay required six operator manipulations and approximately 45 minutes to perform, Biosensors Team has developed a one step process called the FASTube to simplify and accelerate the ECL assay without any loss of sensitivity. An immunomagnetic one-step ECL sandwich immunoassay was developed that provides simple to perform yet sensitive identification of antigens in biological samples. All assay constituents needed (except the antigen to be detected) for one assay are lyophilized in a standard ORIGEN test tube to simplify sequential assay chemistries for ease of use.[38] An operator with less than one day's training can use the system and identify antigens within 15 minutes.

#### *4.3.2.13 Threshold*

The Threshold system is produced by Molecular Devices. Basically, it works by incubating the sample containing the analyte of interest with the appropriate binding proteins or oligonucleotide probes. The analyte will be bound by the binding proteins or hybridized to the probes to form a complex. The sample mixture is then filtered through a membrane where the analyte complex is captured and separated from the sample. The captured analyte complex will contain enzyme proportional to the amount of complexed analyte. The membrane is then inserted into the Threshold reader, which contains the substrate urea and the light-addressable potentiometric sensor. Inside the reader, urea is hydrolyzed to urease producing a pH change in a microvolume of less than one microliter. Urease activity from eight different samples is simultaneously detected by the sensor during a 90-second kinetic measurement, which is then processed by the system's software and quantified.

#### *4.3.2.14 Molecular Polymeric Imprints*

Antibody based biosensors have attracted considerable attention in the past two decades, however such devices often lack storage and operational stability because they are based on a fragile biological recognition element - an enzyme or antibody. An emerging technology called molecular imprinting could provide an alternative. This technique leads to highly stable synthetic polymers that possess selective molecular recognition properties because of recognition sites within the polymer matrix that are complementary to the analyte in the shape and positioning of functional groups. Some of these polymers have high selectivities and affinity constants, comparable with naturally occurring recognition systems such as monoclonal antibodies or receptors, which show potential for diagnostic assays.[39]

Molecular imprinting is becoming increasingly recognized as a technique for the ready preparation of polymeric materials containing recognition sites of predetermined specificity. The technique of molecular imprinting allows the formation of specific recognition and catalytic sites in macromolecules by the use of templates. Molecularly Imprinted Polymers (MIPs) have been used in an increasing number of applications. Several useful reviews of the molecular imprinting field have recently been published. Of special interest to the developers of diagnostic assays is the potential use of MIPs in sample preparation as antibody or receptor binding site mimics in recognition and assay systems, and as recognition elements in bio-sensors.

In molecular imprinting, intermolecular complementarity is introduced in the form of polymerizable monomers that are allowed to associate with a molecular template before extensive cross-linking 'locks in' the template molecule's shape, size and topological distribution of reactive sites in the polymer matrix. Extraction of the template subsequently exposes the polymer 'cast' that can be used as a receptor for rebinding the original template molecule. Most imprint studies to date have

employed bulk polymerization methods to create a macro-porous block polymer that is crushed, ground and sieved to a desired particle size. More recent studies are focused on methods for forming molecular imprints on the surface of a polymer matrix, which would be more compatible with imprinting larger biological molecules or whole cells. Examples include:

- formation and imprinting of polymer beads,
- photo-lithographic processes for 'etching' molecular shape/size onto a reactive surface,
- electro-polymerization of thin films incorporating molecular templates, and
- vacuum formation of polymer films over biological molecules.

Applications include chromatographic and chiral separations, solid phase extractions, antibody-mimics, sensor coatings, and adsorbents.

It is likely that this technology is not sufficiently mature for consideration in the P3I of currently fielded systems.

To make the imprinted polymer, the molecule to be imprinted is dissolved in an organic solvent, such as chloroform, with a functional monomer, a cross-linking monomer, and a polymerization initiator. The functional monomer is chosen to have a chemical functional group that will interact and pre-associate with the imprint molecule. Ionic, hydrogen bonds, hydrophobic, metal coordination, and covalent bond interactions are typical. Following pre-association, polymerization occurs by UV irradiation or mild heating. Once the solid polymer has formed, it is ground in a mortar and pestle and sieved to obtain a desired size, and the print molecule is extracted by incubation in a solvent capable of disrupting the specific interactions between the imprint molecule and the polymer. This extraction step often involves including an acid or base in the solvent. What remains are rigid stable polymer particles that have pockets complementary in shape and electron density to the imprint molecule. Shape complementarity results in high specificity while multiple interactions between the polymer and individual imprint molecules yield high affinity.
Molecular polymeric imprints offer the advantages of having much greater operational and storage stability. They are chemically inert, require much less time to produce than monoclonal and polyclonal antibodies and no mammals or animals are required for their production.

### *4.3.3 DNA-based Identification*

The characteristics of all living organisms are essentially determined by information contained within DNA are inherited from their parents. The molecular structure of DNA can be imagined as a zipper with each tooth represented by one of four letters (A, C, G or T), and with opposite teeth forming one of two pairs, either A-T or G-C. The letters A, C, G, and T stand for adenine, cytosine, guanine, and thymine, the basic building blocks of DNA.[40] The unique DNA structure of each organism can be used to identify pathogens and biological warfare agents.

DNA-based identification technologies capitalize on the extreme selectivity of DNA and Ribonucleic Acid (RNA) recognition. Nucleic acid probes, engineered single strands of RNA or DNA, bind specifically to strands of complementary nucleic acids from pathogens. These probes and their binding can be detected directly or by tagging with an easily detected molecule that provides a signal. The design of a probe can be highly specific if there is a good fit to a pathogen-unique region of the target nucleic acid, or it can provide more generic identification if there is a fit with a region of nucleic acids conserved among several related pathogens. The sensitivity of these hybridization assays for bacteria is between 1,000 and 10,000 colony-forming units; improved sensitivity is an important area of research. Since the reaction is in real time, the time-consuming part of the method relates to sample preparation and the time required to detect the signal.

The main advantages of nucleic acid-based methods are:

- universality (all living organisms have DNA and/or RNA),
- specificity (every type of organism has some unique sections of DNA or RNA),
- sensitivity (with amplification, very small amounts can be detected),
- adaptability (base sequences common to several microbes, or even a whole class of microbes, can be used as probes), and
- multiplexing capabilities for a host of different microbes (a sample can be probed for many different sequences simultaneously).

Disadvantages of this technology include difficulty in isolation and "clean-up" of DNA samples, degradation of the nucleic acid probes, and interference from related sequences or products. These are important obstacles to be overcome, even after specific and accessible target sequences are identified and probes constructed.[41]

#### *4.3.3.1 Polymerase Chain Reaction (PCR)*

The chemistry of PCR depends on the complementarity of the DNA bases. When a molecule of DNA is sufficiently heated, the hydrogen bonds holding together the double helix are disrupted and the molecule unzips or “denatures” into single strands. If the DNA solution is allowed to cool, then complementary base pairs can reform (renature) and the original double helix is restored.

The polymerase chain reaction (PCR) is a test tube system for DNA replication that allows a "target" DNA sequence to be selectively amplified, or enriched, several million-fold in just a few hours. Within a dividing cell, DNA replication involves a series of enzyme-mediated reactions, whose end result is a faithful copy of the entire genome. Within a test tube, PCR uses just one indispensable enzyme - DNA polymerase - to amplify a specific fraction of the genome.

During cellular DNA replication, enzymes first unwind and denature the DNA double helix into single strands. Then, RNA polymerase synthesizes a short stretch of RNA complementary to one of the DNA strands at the start site of replication. This DNA/RNA heteroduplex acts as a "priming site" for the attachment of the DNA polymerase, which then produces the complementary DNA strand. During PCR, high temperature is used to separate the DNA molecules into single strands, and synthetic sequences of single-stranded DNA (20-30 nucleotides) serve as primers. Two different primer sequences are used to bracket the target region to be amplified. One primer is complementary to one DNA strand at the beginning of the target region; a second primer is complementary to a sequence on the opposite DNA strand at the end of the target region.

To perform a PCR reaction, a small quantity of the target DNA is added to a test tube with a buffered solution containing DNA polymerase, oligonucleotide primers, the four deoxynucleotide building blocks of DNA, and the cofactor $MgCl_2$. The PCR mixture is taken through replication cycles consisting of:

- one to several minutes at 94-96 degrees Celsius, during which the DNA is denatured into single strands;
- one to several minutes at 50-65 degrees Celsius, during which the primers hybridize or "anneal" (by way of hydrogen bonds) to their complementary sequences on either side of the target sequence; and
- one to several minutes at 72 degrees Celsius, during which the polymerase binds and extends a complementary DNA strand from each primer.

As amplification proceeds, the DNA sequence between the primers doubles after each cycle. Following thirty such cycles, a theoretical amplification factor of one billion is attained.

Two important innovations were responsible for automating PCR. First, a heat-stable DNA polymerase was isolated from the bacterium Thermus aquaticus, which inhabits hot springs. This enzyme, called the Taq polymerase, remains active despite repeated heating during many cycles of amplification. Second, DNA thermal cyclers were invented that use a computer to control the repetitive temperature changes required for PCR.

Following amplification, the PCR products are usually loaded into wells of an agarose gel and electrophoresed. Since PCR amplifications can generate microgram quantities of product, amplified fragments can be visualized easily following staining with a chemical stain such as ethidium bromide. While such amplifications are impressive, the important point to remember is that the amplification is selective - only the DNA sequence located between the primers is amplified exponentially. The rest of the DNA in the genome is not amplified and remains invisible in the gel.[42]

Commercially available rapid PCR systems are available that can amplify and identify known DNA sequences within 30-35 minutes.

#### 4.3.3.2 Combinatorial Peptides

Antibodies represent one of the most specific of biological molecules capable of recognizing and binding to any given molecule or organism (toxin, virus, bacteria). They can be relatively non-specific (polyclonal) or uniquely specific (monoclonal) for a given target. Native antibodies are large complexes of 2-300,000 daltons molecular weight and, like most biological molecules, will denature or lose their specific properties if not stored and used under specific environmental conditions. The specific recognition site of an antibody, which recognizes the specific epitopes on the target antigen, is only a fraction of the whole molecule. Advances in genetic engineering have allowed researchers to isolate and clone antibody fragments which at least retain, and often improve, the molecular specificity of the whole antibody while reducing its size. Genetic technology also has developed the ability to develop combinatorial libraries of the gene codes controlling antibody synthesis, thus allowing for the construction of super gene libraries in which all possible combinations of antibody components can be screened for binding to a target ligand.

It has been shown in a few cases that the specific cell-surface epitope that an antibody recognizes and binds to can be mimicked by a linear peptide chain of only 4-7 amino acids. This not only greatly reduces the size of the molecule to be used as a reagent, but largely eliminates the storage and user requirements for maintaining the secondary and tertiary structure of the native antibody. The technology of phage display has been employed to construct combinatorial libraries composed of billions of randomized peptide sequences. The libraries can be rapidly screened against any given antigen targets to identify clones that bind the antigen. The DNA of positive clones are then sequenced and a consensus peptide sequence obtained and synthesized to test for binding affinity and specificity. In addition to identifying short peptide sequences that can substitute for native antibodies, the peptide libraries also can be used to map the active epitope sites on an antigen. These epitope mimetics can be employed as substitutes, or positive controls, for the actual antigen in laboratory and field tests of detection devices without resorting to extensive individual protection against pathogenic organisms.

- Applications: Reagents for biological detection, antibody substitutes, epitope mimetics (positive controls)
- Advantages/Benefits: Smaller molecules are more stable, easier to produce, lower cost, reduction of logistics tail, rapid screening against new threats, greater control over device patterning.

### 4.3.4 Raman Scattering

Raman scattering (or Raman Effect) is an optical property that can be exploited to identify known biological and chemical agents. Transparent substances, if illuminated with strictly monochromatic light, can exhibit in their spectrum in addition to the strong line of the incident frequency (the only one to be expected according to classical theory) fainter lines of lower and higher frequencies. The presence of the new frequencies is explained by assuming that light is composed of photons that either lose energy to molecules or gain energy from excited molecules, as they pass through the substance. This theory was posited by Raman in 1928.

In laboratory application, a laser illuminates the sample on a substrate and generates Raman scattering. A spectrometer produces Raman spectrum “signatures” or “fingerprints.” The fingerprint of the sample is matched against a library of known fingerprints. The sample is identified by point-by-point matching of the sample with the library at all wavelengths.

A fieldable instrument would appear similar to a large microscope coupled to a laptop computer. The laser would illuminate the bio-concentrator array through a dichroic beamsplitter and a focusing lens. The back-scatter passes through the splitter, through collimation optics, through an imaging Raman spectrometer, and finally to a 2-dimensional charge coupled device array sensor. At each charged coupled device pixel, unknowns are identified in the spectral domain by matching the unique spectral fingerprint against a library of known fingerprints. At key wavelengths, unknowns are identified in the imaging domain by the pattern of biomolecules to which they bind and the intensities of the signal at each pixel. Hyperspectral data in both the image and the spectral domains are used to identify each target found in the sample using sophisticated matching algorithms. Such an instrument would potentially be capable of

identifying any known biological or chemical target.

The Raman scattering can be significantly enhanced through the selection of a carefully structured substrate (Surface Enhanced Raman Scattering or SERS). Various gratings, textured and colloidal metals and fractal/microcavity composites have been used in SERS.

## *5.0 CURRENT BIOLOGICAL DETECTION SYSTEMS*

Biological detection systems are designed to protect personnel operating on the ground, at sea, or in the air. Their goal is to provide a real-time capability to detect, identify, locate, quantify, warn, and report biological threats to allow military forces with enough of an early warning to avoid contamination. Chemical warfare agents tend to act quickly so rapid detection is needed to prevent unwanted exposure. Biological agents act more slowly but can affect wider areas because of increased toxicity. No single sensor detects/identifies all biological agents of interest. Providing a timely and effective warning remains a problem. While biological agents are extremely complex and large in comparison to chemical warfare agents, they are only made up of a very limited number of unique building blocks. In general, this means the DoD and DND have to either:

- exploit the 2- and 3- dimensional configurations of biologics (e.g., using antibodies, gene probes/primers, and possibly chromatography),
- use fairly generic detection/identification technologies like fluorescence or size and shape analysis, or
- process the supra-molecular BW agents into more manageable sizes to allow generic detection/identification by chemical warfare type technologies (e.g., IMS and MS).

Biological detection technologies are in a much less mature stage of development than chemical detectors. At present, stand-off biological agent detection systems are in early stages of development, and will not be ready for deployment for many years. Current biological agent detection systems are large, complex, expensive, and subject to false alarms. They can identify only a limited number of biological agents and only after exposure. Sensitivity, selectivity and durability of these detection technologies require improvement. These current systems require substantial power for operation, some requiring the use of dedicated generators.

Many biological detectors use expensive and sensitive reagents, which are a significant logistics burden on the user. Identification depends on having the correct reagents. Recent U.S. Congressional testimony stated that all former Soviet weapons used modified organisms and that current reagents would not detect these products. [43]

Most biological detection systems have significant support requirements. Though the Services are striving for systems that are more autonomous and more automated, at present, many of the developmental and in-service biological agent detection systems are labor intensive due to the use of wet chemistry and sensitive (hence costly) reagents. Some currently fielded systems must be manned continuously by specialized personnel.

There continues to be a large gap between the lethal threat aerosol concentration and limits of detection of current equipment. Both detection and identification systems require high volume, high efficiency collectors to sample the air and present a sample to the detector. The weight and power consumption of these collectors is often a critical problem in the design of affordable or deployable detectors. Smaller, lighter, more efficient collectors would be a definite enhancement to the overall system.

Further research into sample collection and processing is required for fieldable biological detection systems. For instance, the CIBADS samples 600 to 1000 liters of air per minute to deliver a 10-20 ml air or 1-2 ml liquid sample to the detector. With concentrations as low as ten Agent Containing Aerosol Particles per Liter of Air (ACPLA), it is necessary to sample hundreds of liters of air (or at least tens of liters) to ensure there is a good statistical probability of there being a significant number of particles per sample. At ten ACPLA, there is a statistically significant probability that there will not be agent-containing-particles in any one liter of air.

Current detectors available are stand-alone systems that lack connectivity to the Services' command and control networks, a real issue for military commanders leading a joint force of U.S. and allied troops. Many CB experts believe that the integration of command and control systems with CB sensors is essential for the battlefield and that the sensors have not been given enough bandwidth or space. There is a critical need for battlespace information management to provide wide area coverage. It is not enough to know that a point detector alarm has been triggered. The system must present meteorological conditions, topography, predictions, etc. and must fuse data from many

other components (biological and non-biological sensors).

Personnel responding to, managing or investigating a biologically contaminated scene cannot sufficiently detect, identify, and determine the extent of hazardous materials in the environment. In addition, no adequate means exist to detect biological agents within containers or packages non-intrusively or remotely.

Cost is a major impediment to both military and non-military adoption of BW detection systems. The cost of systems to the military must decrease before military users can create networks of sensors. The cost of these systems will need to come down substantially before domestic preparedness operations and commercial users in such areas as Washington, DC, or Toronto, Ontario, could afford to buy the systems in the quantities that they would require to be effective.

### *5.1 U.S. System Development Process*

The U.S. has adopted a process of developing biological detection systems through prototype demonstrations. The DoD is implementing this approach through the use of Advanced Concept Technology Demonstration (ACTD). As described in the ACTD homepage, the concept of ACTD is to exploit mature and maturing technologies to solve important military problems. The reasoning behind using such programs is that facing declining budgets, significant changes in threats, and an accelerated pace of technology development have challenged the military's ability to adequately respond to rapidly evolving needs. In addition, the global proliferation of military technologies, resulting in relatively easy access to these technologies by potential adversaries, has further increased the need to rapidly transition new capabilities from the developer to the user.

ACTDs emphasize technology assessment and integration rather than technology development. The goal is to provide a prototype capability to the warfighter and to support him in the evaluation of that capability. The warfighters evaluate the capabilities in real military exercises and at a scale sufficient to fully assess military utility.

ACTDs are designed to allow users to gain an understanding of proposed new capabilities for which there is no user experience base. Specifically, they provide the warfighter an opportunity:

- to develop and refine his concept of operations to fully exploit the capability under evaluation;
- to evolve his operational requirements as he gains experience and understanding of the capability; and
- to operate militarily useful quantities of prototype systems in realistic military demonstrations, and on that basis, make an assessment of the military utility of the proposed capability.

New and existing technologies are evaluated for incorporation into the biological defense programs through the jointly sponsored Joint Field Trials (JFTs). They provide developers with an opportunity to test and develop their components, which are then evaluated by an analysis team. The cost for DoD to sponsor a JFT has varied but ranges between $800K to $1.2M per trial. If a technology is deemed feasible as a result of its performance in the JFT, it is considered for the Technology Transfer Program, a program that JPO-BD uses to fast track those technologies that demonstrate the best value into their programs. In effect, the Technology Transfer Program is replacing the Program Definition and Risk Reduction (PDRR) phase that traditional acquisition programs use. The program allows the JPO-BD to accelerate the fielding of critical biological defense systems to U.S. forces. The focus is on technologies which demonstrate superior performance, reduce total ownership cost, and have horizontal integration potential for common critical components that are applicable to a broad range of detection programs. To reduce acquisition time, these components are then matured for integration into detection systems. These supporting programs aid the other development programs, which are the Fielded Systems, Current Developments and Future Developmental Systems.

The DoD has used these programs to focus on (in the near term):

- Collector/Concentrators – The goal here is to develop a high efficiency, low power consuming collector/concentrator capable of delivering a detectable level from a low concentration aerosol.

- Generic Detectors – non-wet chemistry – high performing, small, low power consuming dry detectors are key to ensuring that the military forces don't miss an unorthodox BW agent attack. They also are key to reducing the overall size and logistics burden of the entire detection system.
- Dry Identification Technologies –optical stand-off technologies like Light Detection and Ranging (LIDAR), fusing radar signals with an intelligent warning algorithm, improving methodologies for analyzing physical aerosol signatures, miniaturizing and ruggedizing detectors, and exploiting the power of networked systems. There is a big push to examine how to integrate optical stand-off with other technologies.
- Reagents – Antibody and gene-based identification systems are the current state-of-the-art but there is also focus on developing reagents for new and emerging threat agents and in exploiting cutting edge molecular engineering techniques to improve the current reagent sets to make them more sensitive, faster reacting and more specific.

### *5.2 Canadian System Development Process*

The DND uses similar type programs to those being used in the U.S. to develop their biological detection systems. The DND Defence Industrial Research (DIR) Program is a $C4M/year Defence Research and Development Canada (DRDC) program directed at strengthening and supporting the Canadian defense industrial base through the provision of financial and scientific support for eligible industry-initiated research projects which have sufficient defense-relevance to Canada and/or its allies. The objective is to stimulate research and innovation in the Canadian defense industrial base, thereby enhancing its ability to share in the development and supply of materiel and equipment to meet Canadian, North American Treaty Organization (NATO), and other allied defense requirements. The DIR Program provides up to a maximum of 50% of eligible project costs, typically to a limit of $C500K per project. Projects are selected on the basis of technical merit, defense relevance, and the promise exhibited for the stimulation of productivity, exports, and economic growth in Canada. The support of the Canadian Forces (CF) is required, and an officer of the CF is required to champion the support of each DIR project with respect to defense relevance. The scope of projects supported is from the laboratory to the experimental model or proof-of-concept stage. Through the DIR Program, DRDC has recently funded two biological detection related DIR projects – the development of a proof of concept for the miniaturization of fluorescence aerosol particle sizing technology at Computing Devices Canada funded at $C500K and the development of BioAlloy at IatroQuest, also funded at $C500K.

Another Canadian program that aids in funding research for implementation in biological detection systems is the Technology Investment Fund, a $C6M annual internal competitive program available only to DRDC researchers. It sponsors three-year term blue sky R&D projects in any of the DRDC client capability groups. There are currently two biological agent detection projects underway – a $C1.4M DNA probe project (basic PCR/gene probe) at Defence Research Establishment Suffield (DRES), and a peptide mimetic/medical countermeasures project also at DRES.

A third Canadian program for system development is the Technology Demonstration Program (TDP), which is geared to demonstrate technologies fostered by DRDC and Canadian industry in the context of real and potential future CF capabilities, concepts, doctrine, operations and equipment. The TDP is aimed at concept development and evaluation for force design purposes and is therefore typically not focused on hardware development. Projects can be proposed by DND organizations, other government departments, defence agencies of allied nations and Canadian industry. TDP projects are typically 3-4 years in duration and are necessarily fast paced to ensure relevance of the program results and access to state-of-the-art concepts for operational deployment. Projects are typically collaborative in nature and usually require some form of meaningful investment by all stakeholders.

In the following sections, current U.S. and Canadian biological detection systems in use or in the advanced development stage are described.

### *5.3 U.S. Detection Systems*

This section discusses the current U.S. biological detection systems, highlighting information on their costs and benefits. Section 5.3.1 through

Section 5.3.10 highlights systems that are managed by the JPO-BD. Much of this information was included as part of the JPO-BD Assessment of Biological Warfare Detection. Section 5.3.11 through Section 5.3.14 addresses other biological detection systems fielded or under development. The following figure depicts the U.S. joint biological detection strategy.

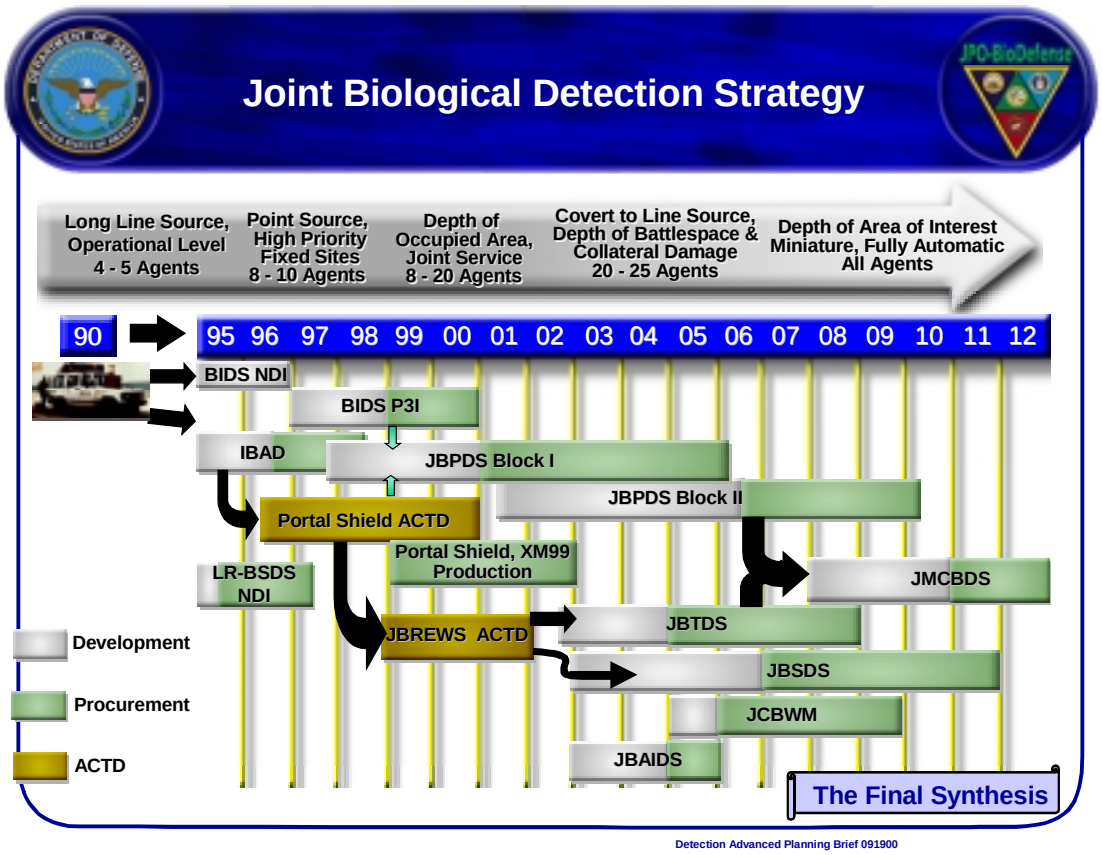


**Figure 5.1, Joint DoD Biological Detection Strategy**[44]

### *5.3.1 Interim Biological Agent Detector (IBAD)*

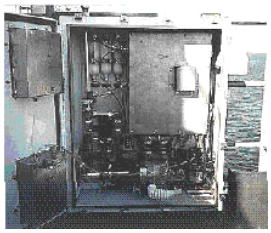

**Figure 5.2, IBAD**

The IBAD is a shipboard semi-automated point detector system for use in blue-water operations. The system is composed of a combined particle counter/sizer – wet cyclone sampler mounted on the forecastle of the ship, and HHAs that are employed manually inside the ship. The particle counter continuously monitors the air for a significant rise in particulate concentrations. If a significant rise over background is detected, the instrument will automatically collect an aerosol sample, and alert the ship's damage control center of the need to collect the sample and screen it using HHAs. A positive identification on the HHAs results in a system-level positive detection.

Compared to the BIDS, Portal Shield and JBPDS, the IBAD is a fairly simple device, but the IBAD's operating environment is also much less complex than the others. The particulate background in blue-water environments is fairly constant, and this enhances the value of aerosol concentration changes for trigger/detection. Also, false alarms (or more accurately false trigger/detections) are more tolerable on a ship that is collectively protected than for an entire air base's population, or an Army division that has to put its troops into degrading protective gear. This system's drawbacks are that it has no true generic detection capability, it is not fully automated, and its effectiveness is greatly reduced by the background clutter common in ports.

The U.S. Navy deployed the first IBAD in 1994 aboard the USS LaSalle, and 19 more IBADs have been deployed with the fleet since then. A total of 20 of these interim systems have been built and 15 are currently shipboard.

The IBAD trigger-collector system weighs about 200 pounds, and occupies a volume of 7.5 cubic feet. Estimated unit price is $165K. Estimated daily operating costs for this system are unavailable.

### *5.3.2 Biological Integrated Detection System (BIDS)*

**Figure 5.3, BIDS**

The U.S. Army's BIDS consists of a shelter (S-788 Lightweight Multipurpose Shelter) mounted on a dedicated vehicle (M1097 High Mobility Multipurpose Wheeled Vehicle (HMMWV)). It is equipped with a biological detection suite employing complementary technologies to detect large area biological attacks. The system includes a trailer-mounted 15-kilowatt generator (PU-801) to provide electrical power. To ensure uninterrupted operation for at least three days, the complete BIDS system also includes a second HMMWV that is used as a support vehicle (to carry additional spares and repairs, and to courier suspect samples to a collection point). It also carries two of the BIDS four-man crew.

To meet the immediate need for a BW detection capability, yet take advantage of maturing technologies, the BIDS has taken an evolutionary acquisition approach. The first version of the BIDS, the NDI version, consists primarily of commercial off-the-shelf items. Technologies within the NDI system's bio suite include: aerosol sizing/counting, bioluminescence, flow cytometry, and immunoassay technologies. The NDI system is completely manual. The follow-on system to the BIDS NDI is the BIDS P3I. The P3I offers an expanded, semi-automated detection/ identification capability. Instead of straight aerosol counting and sizing, the P3I uses a device that looks for both aerosol size and count, and biological fluorescence. The P3I also replaces the bioluminescence device with a pyrolysis-tandem MS instrument, and the manual immunochromatic instrument is replaced with an automated instrument, the biological detector.

The BIDS NDI costs approximately $1M per system, and the BIDS P3I costs about $1.2M per system. The estimated operating cost of the BIDS NDI in peacetime is $800.00 per day per system, and in wartime $1425.00 per day per system.

The first BIDS was fielded to the U.S. Army 310th Chemical Company (Reserve) in 1996. The second BIDS, equipped with the BIDS P3I, was fielded to the 7th Chemical Company (Active) in October, 1999. A final BIDS has yet to be delivered.

The BIDS, as mentioned above, is designed for defense against the most catastrophic type of BW attack - a long line source attack. The doctrinal employment concept for the BIDS is to deploy one U.S. Army Company of 35 BIDS vehicles to an U.S. Army Corps. The BIDS systems are then placed throughout the Corps' area to create a wide area sensor array/network. Any detection is reported directly to the U.S. Army Company headquarters, which is collocated with the Corps (or Joint Task Force (JTF)) Headquarters. The team, consisting of the BIDS Company Commander, Corps Chemical Officer, and Corps Surgeon, then determine if, in fact, a BW attack has taken place (as opposed to the single system alert being due to local fluctuations – a false positive). If the determination is that an attack has occurred, then appropriate warning and post attack actions are executed. The BIDS' low density on the battlefield, and its central control at the theater or JTF level, makes it an Operational-Level Detector.

While each individual BIDS system is very robust in itself, the fact that so few systems are used to monitor such large areas means that localized, point BW attacks may go undetected. Of course, several of the BIDS can be employed at a single high priority site to provide coverage of that site, but there remains the high manpower cost of covering that site (i.e., four operators per system).

### *5.3.3 Long Range Biological Stand-off Detection System (LR-BSDS)*

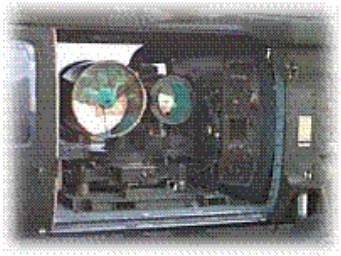

**Figure 5.4, LR-BSDS**

Just as many military systems employ the concept of "defense-in-depth," biological detection can be viewed as providing detection at different physical and operational levels. In this context, the LR-BSDS NDI performs at the outer edge of the detection environment, providing perhaps the earliest warning of biological attacks against the largest echelon of U.S. forces. As a corps asset, the LR-BSDS is flown as close to the forward-line-of-own-troops (FLOT) as is safe and practical (the system is designed to be flown in a UH-60 helicopter). The long range of the system (30 kilometers or more) allows detection of crippling long-line source attacks before the agent has reached and affected U.S. forces, preventing what could be a substantial negation of U.S. military capability. The LR-BSDS NDI is currently the only system supporting ground forces that can tactically reach out and detect threats beyond the FLOT, and field tests have demonstrated its ability to detect line-source threats and to distinguish those threats from other atmospheric phenomena. Some preliminary simulation results indicate that the LR-BSDS may also be effective against other types of releases, such as point releases of large amounts of agent upwind of U.S. forces, given appropriate operator training. The LR-BSDS is the first generation attempt at a detect-to-warn capability.

The LR-BSDS does not discriminate biological material - it can only warn that it detects a suspicious man-made aerosol cloud. Other systems, such as the BIDS, will be required to actually determine that there is agent present once the cloud reaches U.S. forces. However, the warning time afforded by the LR-BSDS can allow U.S. forces to adopt protective measures before any lethal exposures have been incurred. Because of its limited numbers and concept of operations, the LR-BSDS is not intended to be used to detect agent releases that originate in U.S. force areas, such as tactical missile attacks

on U.S. installations; complementary systems are required for those types of attacks.

The LR-BSDS NDI was fielded to the 310th Chemical Company along with the BIDS NDI systems. This system is non-eye-safe out to a distance of 30 kilometers, and requires two operators in the helicopter to control the system, and to manually interpret the laser return data on the system's computer screen.

The per-unit cost of the NDI LR-BSDS is $1.046M.

### *5.3.4 Portal Shield Airbase/Port Biological Detection System*

The objective of the Portal Shield ACTD is to evaluate the military utility of a biological detection network capability and to develop operational procedures for that capability. An additional objective is to provide a residual capability to detect, warn, dewarn and presumptively identify a BW attack on a high priority fixed site. The impetus for the Portal Shield ACTD was the realization that U.S. enemies were arming themselves with a variety of BW delivery systems and not just aerial long-line source systems. That meant that the U.S. had to have a system that could affordably and capably provide detection for fixed sites against small-scale releases; a job for which the current detection systems were not designed.

The Commanders in Chief (CINCs) of both Pacific Command (PACOM) and Central Command (CENTCOM) are sponsoring the ACTD. In January, 1999, the Portal Shield passed a Milestone III decision to go into production to meet a directed buy requirement for 70 additional sensors above the ACTD's requirement. The Portal Shield system is the initial attempt to fulfill the requirement of providing biological protection for Ports and Airfields of Embarkation/Debarkation in support of force projection.

The Portal Shield system is a fully automated system made up of a number of components in order to give the fixed sites a robust biological detection capability. The network itself consists of six or more sensor systems (the typical site requires between 12 and 20 sensors). The Portal Shield Mark III sensor is the heart of the system. The sensors are all linked to a central command post computer that monitors the operational status of the sensors, controls the networked sensors, evaluates network data to determine if a BW attack has occurred, and alerts the operator to a BW detection. The command post is also loaded with decision-aid algorithms to assist in protective posture decisions, and can interface with the Joint Service Warning and Reporting Network (JWARN). The Portal Shield sensors and command post also allow direct integration of a site's chemical detection equipment (e.g., the M22 Automatic Chemical Agent Detector Alarm (ACADA)) into the Portal Shield network. With this capability, the site is able to monitor all of their CW detection equipment remotely by a single operator. Other ACTD leave-behinds are: tested concept of operations, data on half-face particulate respirators that may be used for post attack respiratory protection, a biological decontamination system for the Portal Shield sensors, ELISA kits for on-site back-up identification, and DoD Biological Sampling Kits. In order to survive environmental extremes, such as temperature and humidity, the systems need to be housed in an environmentally controlled shelter.

The Portal Shield ACTD was one of only two programs to transition to full-scale production. An additional 19 critical sites (23 total) were selected by the Joint Staff to receive the XM 99 Portal Shield systems. There are currently eight sites fielded, with 15 sites remaining to be fielded. A total of 167 systems have been manufactured.

According to JPO-BD representatives, the Portal Shield program has yielded invaluable information and lessons learned on biological detection. Portal Shield has also maximized the modular design concept, completely obviating the need for tools for operator level maintenance. The modular design also ensures that the U.S. will be able to upgrade the Portal Shield in the field as better technologies become available. Finally, this program, through its numerous deployments, has greatly accelerated the U.S. understanding of the operational requirements of biological detection.

The Portal Shield's network algorithm uses both aerosol count and meteorological data (wind speed and direction) to determine the presence of a suspect aerosol cloud. The algorithm looks for a significant rise in at least two particle counters, and then uses the wind speed and direction data to determine if the particle data could correspond

to a notional aerosol attack. If the two triggers correlate, then the command post will direct the sensors to evaluate the suspect samples using their automated assay readers. Using its network algorithm, Portal Shield dramatically drives down operational costs due to consumables.

Even though the assays have demonstrated a false positive rate of less than 0.5% in actual operational environments, the algorithm must see at least two positives for the same agent before it will sound an alarm. Requiring a positive identification on two independent assays theoretically drives the system-level false positive rate down to 0.25%. In practice, after having gone through over 10,000 assays in the Portal Shield system, the U.S. has had zero system-level false alarms.

A current weakness of the Portal Shield system is that it does not contain a generic biological detection capability. Also, it is not ruggedized to the point that it can be employed for mobile detection.

Each sensor system is $170K. The daily support cost on a per sensor basis is between $191.00 (when the system is operated in "smart mode"), and $262.00 (when the network is run in random sample mode). The daily support costs include repair parts, consumables, and contractor logistics support costs.

### *5.3.5 DoD Biological Sampling Kit (DoD BSK)*

The DoD BSK's requirement came from a Portal Shield PACOM warfighter exercise held in July, 1997. PACOM U.S. Air Force (USAF) participants expressed a strong desire for something that would allow them to screen suspect packages and munitions for the presence of BW agents. The JPO-BD recommendation was a simple, pre-packaged kit that contains:

- a panel of eight HHA assays (i.e., able to identify eight different BW agents),
- a dropper bottle of buffer solution,
- two sterile cotton-tipped swabs, and
- an instruction card.

The DoD BSK is the first generation attempt at fulfilling the requirement for a Biological Reconnaissance/Survey Capability.

The DoD BSK can be employed for field screening munitions or munitions fragments that are suspected of containing BW agents, packages that are leaking suspect liquids or powders, suspect terrorist laboratories or weapons materials, and indoor areas where the concentration of agent is expected to be high (e.g., from the indoor release of a BW agent). The kit is *not* to be used for screening soil samples. Since some soil constituents can cross-react with the HHA reagents if present in high enough concentration, heavily dust-laden surfaces should also be avoided for screening with the DoD BSK. Also, the kit is not sensitive enough to detect the minute amounts of precipitate that may fall out from an attack that originated from far away; e.g., a long line source release from several kilometers away.

Currently, the DoD BSK is available for military use from the JPO-BD.

The advantages of the DoD BSK are that it is inexpensive, reliable, easy to use, and the assays in the kit are improved concurrent with the assays in the other detection programs. The assays are part of JPO-BD's horizontal technology insertion effort. Disadvantages of the DoD BSK are that it does not possess a generic detection capability (it is an identifier), and each kit is for one time use only. The current cost of the DoD BSK is $44.00 per kit.

### *5.3.6 Biological Agent Warning Sensor (BAWS)*

**Figure 5.5, BAWS**

The U.S. Army SBCCOM sponsored the BAWS program, and Massachusetts Institute of Technology (MIT) Lincoln Laboratory performed the research and development work. BAWS is part of the point detection Advanced Technology Demonstration (ATD) and was developed for generic detection of threat bioaerosols. In 1999, support for BAWS

development was transitioned to the Joint Biological Point Detection System Block 1 that is managed by the JPO-BD. The current BAWS (BAWS III) system uses a three-channel photonic fluorescence sensor. A laser beam illuminates the sample air stream, and three photomultiplier tubes of 266 nm, 300-400 nm, and 400-550 nm sense reflected photons. An alarm algorithm maps the signal output in terms of fluorescent and elastic UV back-scatter response and compares a 5-10 minute history with the present response. A significant change indicates the presence of a new aerosol cloud at the sensor. The algorithm can classify the change as either interferent (such as dust), a potential bio-aerosol, or as an unknown cloud. If it is classified as a bio-aerosol, the alarm is triggered.

The BAWS does not provide an identification function. The BAWS uses 266 nanometer laser radiation to detect tryptophan. This is different than what is used in the current Canadian FPS system, that uses 355 nm laser radiation to excite nicotinamide adenine dinucleotide compounds (NADH).

### *5.3.7 Joint Biological Point Detection System (JBPDS)*

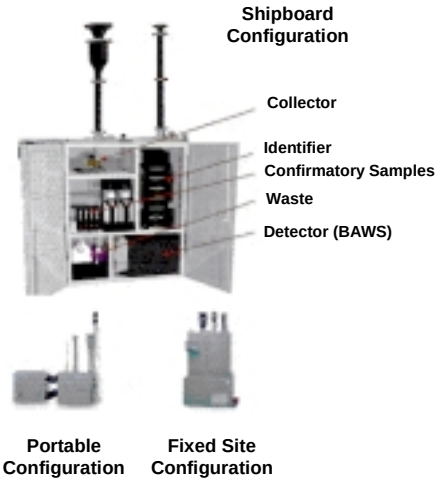


**Figure 5.6, JBPDS**

According to the JPO-BD, the JBPDS is an operational system that provides a common integrated biological point detection suite for use by all Services. It automatically detects and identifies ten BW agents within 20 minutes and offers improved sensitivity over currently fielded systems. It also has an exportable, computer-based training capability. The system is being integrated onto HMMWVs and ships. Portable and fixed-site configurations will enhance joint interoperability and supportability.

The JBPDS is being deployed at air bases and ports, aboard ships, and with mobile forces. Thus, the JBPDS provides a common detection capability throughout the area of operations, and for use by all Services.

JPO-BD had structured the JBPDS for evolutionary, block upgrades. Their original strategy was to build all JBPDS frames in Block I and use Block II to develop better technologies and insert these technologies through modular upgrades. This is no longer the case. Due to the rapid advances in technology and a significant increase in requirements, the DoD has changed its acquisition strategy. The Block I focus, which was recently completed, was on automation and expanding the number of BW agents identified simultaneously from eight to ten. The Block I's, are now expected to remain in the inventory longer but will not be upgraded as required to ensure battlefield viability. Block II systems may be a new design that utilizes dry detection and identification technologies while reducing size, weight and power; and providing increased sensitivity, specificity and full functionality in a single box (trigger, detect, collect and identify). The DoD plans to procure approximately 1100 Block II systems.

JPO-BD representatives assert that the JBPDS represents a significant step forward in BW detection and identification automation, and sensor proliferation across the battlespace. The JBPDS possesses both the sensitivity required of an operational detection system, and the multi-functional capabilities of generic detection, identification, and sample collection. However, the JBPDS is still somewhat limited in where it can go and the roles it can fill by its size and power requirements. For example, the JBPDS is still too large for remote employment (to a large degree the cost of high sensitivity and multiple functions within a single box). The JBPDS is still a detect-to-treat capability.

The estimated per-unit cost of the JBPDS is $350K. Operations and maintenance costs are not yet available as the program has two design opportunities that will influence its life cycle cost. The design incorporates the BAWS UV laser-based detector. Advantages of the BAWS are that its only consumable is electricity, and it is able to respond to all biologics.

### *5.3.8 Joint Biological Remote/Early Warning System (JBREWS)*

The JBREWS, currently being developed as an ACTD, combines a variety of technologies to provide both rapid warning of a biological attack as well as identification and sample collection of agent. The JBREWS is intended to accompany ground forces into theater to provide protection in assembly areas (while forces are temporarily stationary), and to provide temporary protection of ports, airfields, and other critical logistic nodes.

According to developers, the JBREWS ACTD comprises a variety of innovative components that represent the state-of-the-art of their respective technologies:

- **Sample Identification Units (SIUs).** The SIUs continuously sample the atmosphere and check for the presence of biological agent using antibody tickets. Although they are not intended to provide rapid warning, they offer agent identification capability, and can collect samples of agent for more extensive analysis. Because they concentrate what they collect, they can provide notice of lower concentration attacks.
- **The Short Range Biological Stand-off Detection System (SR-BSDS).** The SR-BSDS is included in the JBREWS ACTD to provide remote early warning of a high concentration attack. Because of its stand-off capability, the SR-BSDS may be able to provide warning to troops of some attacks before agent begins to affect them.
- **Reconfigurable Radio Network.** The sensors of the JBREWs communicate through a radio network that is configured automatically, and reroutes transmissions that are blocked by obstacles.
- **Sensor Network Command Post (SNCP).** The SNCP collects and autonomously processes the information provided the sensors and determines whether an attack is occurring. The SNCP can issue an alarm automatically and activate aural or visual alarms on the sensors or can involve a man-in-the-loop to mediate the alarm process, and can prepare and send appropriate NBC messages to higher echelons automatically.

The JBREWS equipment is designed to be deployed and operated by a unit without extensive training or specialized operators, while having as small an impact on the unit's military effectiveness as possible. This allows the JBREWS to be assigned to units and facilities where needed. Quick set up and take down capability allows the system to be moved rapidly into and around a theater as the military situation evolves.

A battalion JBREWS ACTD Deployable Unit Biological Detection System, comprising 25 SUs, five SIUs, and two SNCPs and the supporting equipment is currently estimated to cost $1.5M.

According to JPO-BD representatives, the JBREWS advances the U.S. biological detection program in several ways. By combining rapid warning technologies with identification and sampling technologies into a system intended to operate in the military environment, JBREWS is a further step toward the ideal system. For the first time, maneuver units in the field will have an organic biological detection capability that they can bring with them as they deploy and rely upon to provide warning of biological attacks as they occur. As an ACTD, the JBREWS system will provide valuable lessons learned for future systems with regard to how the JBREWS technologies function in the real world and how best to integrate these systems into operational units.

They perceive the JBREWS ACTD will feed technical and operational lessons learned into the Joint Biological Tactical Detection System (JBTDS) program and the Joint Biological Stand-off Detection System (JBSDS) program.

Noting that point and stand-off detection systems have unique strengths and weaknesses, JPO-BD underscore that the successful application of these technologies may be highly dependent on the environment. Geographic, seasonal and diurnal variation can affect the utility of certain technologies. Single-technology systems will not be capable of fulfilling all the bio detection requirements that doctrinal and requirements documents have prescribed. An optimal mix of networked technologies will allow the U.S. to fill detection requirements that span the battlespace from counterforce actions to providing credible detection for deploying, tactical forces.

### *5.3.9 Joint Modular Chemical and Biological Detection System (JMCBDS)*

The JMCBDS will be DoD's first program for the integration of chemical *and* biological detection technologies into a single system. It is envisioned that the system will be modular and configurable in design. This will allow for maximum flexibility against a highly diversified threat. The program is envisioned to encompass both point and remote sensor capabilities. It will be substantially smaller and lighter than current systems.

### *5.3.10 Deployable, In Theater Laboratories*

Deployable, in-theater laboratories are critical for providing the rapid confirmatory analyses and medical diagnostic support that is crucial for refinement of post-attack countermeasures. The U.S. Army and the U.S. Navy already have limited numbers of deployable laboratories that have been deployed in support of actual contingencies. The U.S. Army's Theater Army Medical Laboratory and the U.S. Navy's Forward Deployable Laboratory – BW Module were both deployed in 1998 in support of Operation Desert Thunder, and both organizations have supported a number of other domestic operations. Both systems are managed through the Services' medical materiel chains, but the JPO-BD detection program must be coordinated with them for several reasons, as noted in the following paragraphs.

This capability is critical to the overall, coordinated biological detection strategy. Samples from the operational and tactical detection systems will go to the theater laboratories for further analyses.

Second, U.S. detection systems use many of the same critical reagents that these laboratories use. The JPO-BD office indicated that it only makes sense to develop these laboratories' capabilities in coordination with the JPO-BD detection systems to ensure both economic procurement, and consistent performance against the same BW agents (that is, coherent battlespace visualization).

Finally, there is a foreseeable convergence in technology applications between both the tactical and operational biological detection systems, and the laboratory instruments. Identifiers, especially the DNA-based systems, are currently being explored for application in rapid, automated medical diagnostic kits. Potentially, the JMCBDS could be used in these laboratories, too.

### *5.3.11 Joint Service Light NBC Reconnaissance System (JSLNBCRS)*

**Figure 5.7, JSLNBCRS**

The JSLNBCRS is a coordinated U.S. Army, USAF and U.S. Marine Corps (USMC) research program to develop improved reconnaissance capabilities for both heavy and lightweight vehicle platforms. Integrating advanced NBC detection and analysis equipment, it will be used by USMC Air-Ground Task Forces, USAF Tactical Forces, and U.S. Army Light Contingency Forces to verify, find, map and mark radiological, biological, and chemical hazards. Two configurations have been proposed – a light system for expeditionary situations and a medium system for armored missions.

The JSLNBCRS consists of a base vehicle equipped with portable and vehicle mounted NBC detection and identification equipment. Major components of the system include:

- Chemical stand-off and CB point detection
- Contamination area marking
- Automated NBC hazard prediction, analysis and dissemination
- Radiation detection
- Global Positioning System (GPS) – contaminated areas digital mapping
- MET – collect and analyze meteorological data
- Communications
- Vehicle intercom system
- Collective protection/environmental controls
- Auxiliary power unit.

Some of the technological challenges of this system that the DoD is trying to overcome

include sensor weight, sensor size, and time to detect/identify. Lighter and smaller sensors that will allow for integration onto a variety of light platforms are desired.

### *5.3.12 Joint Service Chemical Biological Agent Water Monitor*

This agent water monitor system is a cooperative Research, Development Test and Evaluation (RDTE) effort between the U.S. Army, USAF and USMC for the development of a detection system that will detect CB agents in water. Designed to improve upon existing agent water monitor capabilities, the system will feature multi-agent capabilities and operate automatically. Researchers envision that this system should improve both the response time of current water monitoring and purifying capabilities, monitoring automatically with continuous and batch sampling capabilities. The scientists are designing this system to be compact, manportable and easy to use, and to allow for the automatic detection of CB agents at or below harmful levels in water without setting off false positives due to common interferents. Scenarios where this system will be used include source water, water distribution systems, and verification of water treatment.

The agent water monitor system fulfills joint U.S. Army, USAF and USMC requirements and has generated U.S. Navy interest. It is envisioned that this system could have a dual use function in the markets of monitoring civilian water supplies and environmental monitoring.

A market survey has been conducted where 150 candidate technologies were identified. A down selection was then initiated and from that, five technologies are being baseline tested. In FY 2001-02, a breadboard system will be built and tested. An estimated 20,000 units will be required for Joint Service use.

### *5.3.13 Force Medical Protection/ Dosimeter*

The USMC is conducting an ACTD for the force medical protection/dosimeter, a system they envision as an individually worn sampler that will be capable of measuring and archiving exposure levels of CB agents. The system is designed to warn the user, provide real-time analysis of chemical agents, and trap biological agents for later analysis.

### *5.3.14 Joint Service Warning and Reporting Network (JWARN)*

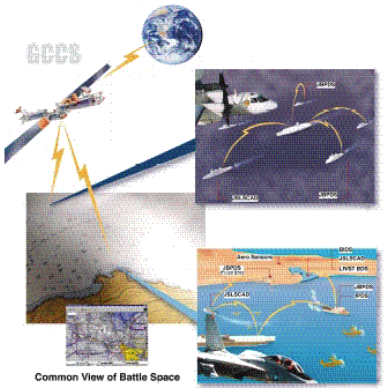


**Figure 5.8, JWARN**

Note: Although JWARN is not a specific biological detection system program, it is a component of the biological warning system. Because the study team has referenced it in the discussions on different detection systems an explanation of its function is included here.

The JWARN is an automated nuclear, biological and chemical information system that is designed to integrate the data from NBC detectors and sensors into the Joint Service command, control communication, computers, information and intelligence systems and network in the digitized battlefield. JWARN is designed to provide the Joint Forces with a comprehensive analysis and response capability. The system is also supposed to provide the Joint Forces with the operational capabilities to employ NBC warning technology to collect, analyze, identify, locate, report and disseminate NBC threat and hazard information. It will transfer data automatically from and to the detector/sensor/network node and provide commanders with analyzed data for decisions.

The JWARN Phase I effort began fielding the first version of software in FY1998. The JWARN Phase II effort was initiated in FY1999 for hardware and software integration onto Service designated platforms and installation at fixed sites.

### *5.4 DND Detection Systems*

Section 5.4 addresses current Canadian biological detection systems. The main CF concept of use for an integrated CB warfare agent detection system is to protect personnel at

high value, fixed assets, such as headquarter areas, field hospitals or airfields. The DND wants a system that is automated and provides near real time detection. The researchers envisioned that such a system should consist primarily of a network of remote point detection systems. That is "sentries" capable of autonomous operation, real time detection, and rapid identification of all threat CB agents. Their goal is to develop a detector that provides a local alarm (i.e., light or bell) and notifies the command and control system. The local tactical alarm will advise those in the immediate area to don protective gear. The command and control input is to allow an appropriate tactical response (this alarm by itself does not justify a strategic response). The desired biological detection system would be able to capture at least two samples - one for tactical analysis (i.e., to allow field medical personnel to initiate prophylaxis) and one for forensic analysis at a national lab.

### *5.4.1 Mobile Atmospheric Sampling and Identification Facility (MASIF)*

**Figure 5.9, MASIF**

MASIF was deployed in the Gulf during the 1991 conflict. The facility was designed by DRES for detection of biological agent attacks, collecting and analyzing aerosol samples and identifying selected biological agents that they may contain. The system consisted of the Mobile Atmospheric Sampling Units (MASU), which are self-contained units deployed as a network around militarily sensitive areas (e.g., airfields, command centres) and which continuously monitored the particulate content of the air. Should a distribution of particles, indicative of a biological agent attack, be detected, an alarm was relayed to a central location and samples were collected for further analysis. The samples were then analyzed in the Mobile Agent Identification Unit (MAGIDU), where the presence and identity of specific biological agents was ascertained using fast DRES-developed assay methods. Upon triggering of the alarm, CF personnel took suitable precautionary measures; upon identification of the specific agent used, further medical treatment was applied, if required.

This system featured:

- Remote unmanned operation of multiple detectors
- Stand-alone or array operation
- Deployable by military transport aircraft and helicopters
- Modular hardware and software for future improvements
- Capabilities for rapid field deployment.

Government officials noted that, coupled with the DRES Chemical Agent Detection System (CADS), the MASIF allowed the CF operating in the Gulf to carry out their duties without having to continuously wear cumbersome CB warfare gear. The protection offered by MASIF also allowed CF to discontinue taking the standard prophylactic medication which, although providing adequate protection, also carried penalties in the form of reduced efficiency and other deleterious side-effects. Some of the technologies employed in MASIF have been put forward by a US/UK/Canada panel on the development of a next generation biological agent detector.

### *5.4.2 FLuorescence Aerodynamic Particle Sizer (FLAPS)*

**Figure 5.10, FLAPS**

DRES built FLAPS for the measurement of biological aerosols. The FLAPS was designed to measure particle size and intrinsic biological fluorescence for each particle in an air stream. As a result, it is able to distinguish, in real time, those particles in air which contain living organisms from all other background particles. It can be used to reliably detect low concentrations of man-made aerosols such as biological warfare

agent clouds and distinguish this from normal background material.

FLAPS 2 is based on the TSI Aerodynamic Particle Sizer™. This particle sizer samples air at a rate of one liter per minute and counts/sizes the particles between 0.5 and 15 micrometers. It achieves this by accelerating the particles through a small orifice and measuring the time to traverse the distance between the two halves of a split red diode laser beam (680 nm). Each particle is detected by light scattering using an avalanche photo diode.

FLAPS 2 adds to this basic particle sizer the ability to measure the intrinsic fluorescence produced by living organisms which contain the bio-active molecule NADH or other similar flavinoid molecules.

This property of living organisms was first measured in particles in a moving liquid stream using flow cytometry; the same property has been measured in a moving particle stream in air. When a particle breaks the red laser beam and is counted, the event triggers a second pulsed UV laser (355 nm) to excite the particle. Fluorescence from the particle (420-580 nm) is then measured by a second photo multiplier.

FLAPS 2 also contains software designed to automatically log all particle and fluorescence data, display a three dimensional plot of the particle size/number/fluorescence, and provide an automatic alarm when an unusual proportion of fluorescent particles is encountered during sampling of ambient air. This software provides the ability for the FLAPS 2 to operate autonomously at a remote location and provide information automatically about dangerous levels of living organisms such as biological warfare agents or infectious diseases.

At recent field trials, FLAPS 2 was able to detect 39 of 40 blind releases of simulant aerosols at a distance of about a kilometer with no false alarms logged over a period of three weeks of eight-hour-per-day operation. In the second set of trials, FLAPS 2 was demonstrated to be the first instrument which could reliably detect as few as ten ACPLA in normal background air samples, and provide an automatic alarm in less than 15 seconds, again with no false alarms.

### *5.4.3 Canadian Integrated Biological Agent Detection System (CIBADS)*

**Figure 5.11, CIBADS**

The CIBADS program was initiated to develop and demonstrate an Advanced Demonstration Model (ADM) capable of integrated, automated CB agent detection, sample collection, and identification. Begun in 1993, the CIBADS project objective was to produce a field-portable integrated CB agent detection system for acquisition by the CF in the year 2000. CIBADS is a point detection system made up of a number of CB Sentry Units linked to a command and control system. To date, the integrated detection, identification, and sentry concepts have been demonstrated and proven during international field trials using biological agent simulants.

The CIBADS Concept Demonstrator was developed at DRES as a mobile laboratory for environmental biological aerosol detection, collection, and identification. CIBADS can detect, in real time, the presence of chemical agents and of living biological agents in an aerosol cloud. DRES representatives pointed out that this is a major advance over previous systems which required several hours to achieve this goal. The design of this system was to provide the capability to detect a broad spectrum of chemical or biological agents in time to allow individuals to don protective equipment, identify the agent in time to initiate appropriate medical counter measures, and collect vapor/liquid samples for verification of the agent.

The CIBADS uses a FLAPS to distinguish biological agent particles from all other airborne particles for biological detection and an ion mobility spectrometer for chemical detection and identification. For biological agent detection, a positive response from FLAPS triggers the collection of a liquid sample for analysis by an Automated Ticket Reader (ATR) which can

subsequently identify the agent. For chemical agent detection, a commercial ion mobility spectrometer is used to detect any vapours and trigger collection of a vapour sample on an absorbent tube. The system is radio-linked to a command and control unit which can accept incoming data from a large number of sentries, plot the position of positive detection events on field maps, and couple the information to hazard assessment tools which predict the downwind hazard and source of the agent attack. The command and control unit also can be used to position detectors and troops to maximize the probability of detection and minimize the probability of troops being affected by downwind movement of agent clouds.

It is capable of autonomous operation for extended periods of time, will automatically detect CB threats and collect aerosol and vapor samples for further analysis. In the final phase of the CIBADS II project, automatic biological identification and advanced planning tools for the military commander, such as downwind hazard prediction and information for consequence management, will be incorporated.

The CIBADS II unit was deployed to JFT II at Dugway, Utah, in 1995 and 1997, to demonstrate the CIBADS concept for integrated biological detection and identification. It has been deployed to coalition operations in the Gulf. In November, 1997 the CIBADS II ADM was deployed to Vancouver, B.C. for the Asian Pacific Economic Conference. This represented the first time that Canada had deployed a CB agent detection system in a counter-terrorism role.

In February, 1998, the CIBADS II ADM was deployed on-board HMCS Toronto in support of the Operation Determination to force Iraq to comply with United Nations Security Council inspection requirements. This represented the first time that the CF deployed a fully integrated CB agent detection system on a naval vessel.

To provide interim an biological detection capability on current operations in areas where a BW threat has been identified, DND procured and fielded two 4WARN systems. 4WARN is a commercial variant of CIBADS II (without chemical detection capability) manufactured by Computing Devices Canada.

#### *5.4.4 CB Sentry*

The CB Sentry Concept Demonstrator is an autonomous biological detector that uses the second generation FLAPS for biological detection and could be remotely operated by radio modem link with data transmitted back to a command and control unit for analysis and alarming. The CB Sentry Concept Demonstrator was deployed to the JFT III in 1996.

#### *5.4.5 CF Biological Agent Detection, Identification and Warning System – Bio Sentry*

DND has a project to define and procure the next generation of biological agent detection systems. The CF Biological Agent Detection, Identification and Warning System will procure four biological detection deployment sets for the CF. One biological detection deployment set will consist of a number of individual detectors that are capable of satisfying the current concept of operations (i.e., automated, near real-time, tactical system for protection of high value, stationary assets). The concept of operations is to have two sets deployed, one in movement and the fourth in overhaul and maintenance or as a spare. The options analysis phase will be completed before March 31, 2001 and the three-year definition phase will start soon after April 01, 2001. Delivery of systems is scheduled to commence in FY 2004/2005. System details are still under development and will be defined and refined in the definition stage.

## *6.0 BIOLOGICAL DETECTION SYSTEM TECHNOLOGY AND INDUSTRIAL BASE*

Funding for CB detection technologies has been on the rise. In the U.S., this area enjoys the support of several budget lines that fund work being conducted in support of this technology focus, including funding allotted for counterproliferation, domestic preparedness, and demilitarization. Forecasters predict that concerns about terrorists or rogue states using CB weapons against the U.S. will greatly increase the funding for procuring biological and chemical detection systems, with some estimates at $800M a year by 2003. The analysts predict the global market for CB sensors will grow nearly seven percent annually through 2005, though they estimated the biological weapon detection market will grow at an even faster rate – twelve percent a year through 2005.

The Canadian and U.S. governments have worked hard to communicate with industry on their NBC procurement plans for the future. SBCCOM holds Advance Planning Briefings for Industry (APBI) annually. The purpose of these APBIs is to provide academia and industry with information on the SBCCOM Research, Development and Engineering Center's (RDEC) mid- and long-term research and development plans and future needs of military items. Their intent is to engage the private sector in open, meaningful dialog to develop a mutual understanding of future military requirements and industry capabilities. SBCCOM personnel have stated that they recognize that industry's access to advance planning and requirements information as well as advice on doing business with the government increases the effectiveness of bids and proposals, fosters competition, helps to surface scientific and technical developments, and increases the productivity of Independent Research and Development (IR&D). In this same vein, the Canadian government has sponsored Industry Days for the private sector to highlight potential opportunities to work with them on CB detection technology and system development.

The NBC industrial base sector is primarily supported by small and medium sized companies. Many companies who manufacture laboratory equipment for other markets also are watching developments in this field, looking at the potential to tailor their instruments to function in a biological warfare agent detection system. In fact, it was difficult for the study team to ascertain what companies were key players in this field, versus companies that were promoting their laboratory equipment but had no real experience in this arena. The study team reviewed the list of companies, laboratories, and academia involved in biological detection technologies they had compiled from open literature sources with the Technical Advisory Panel. From this review, the panel pared down the list to reflect key companies in various biological detection sub-technology categories that they perceive could play a role in next generation detection systems. This revised listing is provided in Table 6.1. This list should not be regarded as all inclusive, but rather as providing a representative sample of the industry based on inputs from the Technical Advisory Panel. Breaking down these technologies into major technology and subtechnology categories generated a lively debate within the Technical Advisory Panel. The study team recognizes that other biological detection technologies developers' viewpoints on technology categorization may vary somewhat from this list, but this was deemed a generic framework from which the study team could focus their data gathering and analysis. We recognize that many of these companies are involved in a number of different technological initiatives and that the category for which they are listed merely reflects that they are considered a major player in that particular category as well.

As time and funding permitted, the study team conducted site visits and phone interviews with a number of these companies, laboratories, and academia. Information on these organizations are included in Appendix F. The level of detail and depth of information varies for each organization based on the information provided by that organization. Some organizations were sensitive about sharing information about their technologies, R&D investments, and future plans due to the fact that they had patents pending, were on the threshold of achieving a technological breakthrough, were about to go public, or were keenly concerned about providing insights that might aid their competitors in securing more of the marketplace base.

**Table 6.1, Representative Biological Detection System Technology and Industrial Base For Major Technology Categories**

| *Major Technology Category* | *Subtechnology Category* | *Company/Laboratory/Academia* |
|---|---|---|
| **Samplers** | Cyclone | Midwest Research Institute<br>Research International |
| | Virtual Impactors | MesoSystems<br>Dycor |
| **Trigger/Detection** | Fluorescence Aerosol Particle Sizing | Computing Devices Canada |
| | Biological Aerosol Warning Sensor | Lincoln Laboratory MIT |
| | PY/GC-IMS | FemtoScan |
| | Flame Photometry | CyTerra Corporation |
| | Size and Shape Analysis | BIRAL |
| | Flow Cytometry | Lawrence Livermore National Laboratory |
| **Identifier** | Mass Spectrometry - ESI | Orbital Sciences Corp |
| | Mass Spectrometry - Time Of Flight | Johns Hopkins APL<br>Orbital Sciences<br>Bruker Daltonics |
| | Capillary Electrophoresis | DRES<br>DOE |
| | Synthetic Ion Channel | DARPA<br>AMBRI |
| | Ion Channel Switch | University of Texas |
| | Tissue Based Biosensors | DARPA |
| | Lateral Flow Assays | Majesco Biologicals Inc. |
| | Fiber Optic Waveguide | Naval Research Laboratory<br>Research International |
| | Surface Plasmon Resonance | Battelle<br>Molecular Devices Corporation |
| | Resonant Mirror | CBD Porton Down |
| | Optical Sensor | Johns Hopkins APL<br>Sensors for Medicine & Science |
| | Electrochemical Luminescence | IGEN International |
| | Threshold | Molecular Devices |
| | Polymeric Imprints | University of Texas |
| | Gene Probe | Cepheid<br>Affymetrix<br>Idaho Technology<br>Argonne National Laboratory |
| | Force Differentiation Sensor | Naval Research Laboratory |
| | DNA Based Recognition | University of Alabama<br>ID Biomedical Corporation |
| | Peptides | University of Texas |
| | Raman Scattering | Biopraxis |

### 6.1 Dual Use Technology Considerations

CB detection technologies have dual use potential in pharmaceutical and medical diagnostics, and monitoring air pollution and air quality in plants, noxious fumes inside enclosed areas, and municipal water supplies. Detection systems are needed for first responders, the military, the U.S. Secret Service, the Federal Bureau of Investigation, fire departments, airports, embassies, and hospitals.

Due to the makeup of the technologies used in the present and emerging detection systems, the biological detection systems reap the benefits of advances being made in various high growth technology areas, including biotechnology, computer technology, display technology, micro electronics, nano technology, communication technology, and low level signal recovery technology.

### 6.2 Current Biological Detection System Inventory Concerns

The Services currently have very low inventories of some biological detection equipment. Some DoD officials have asserted that fluctuations in funding and inter-service disagreements have hampered the DoD's efforts to deploy advanced detectors in the field. This has contributed to a lack of preparation in the technology base.

As cited in the DoD *Chemical and Biological Defense Program Annual Report to Congress*, March, 2000, the Biological Integrated Detection System and the Interim Biological Agent Detector would be inadequate to fulfill current Major Theater War (MTW) requirements – operating in two nearly simultaneous MTWs. New point detector systems and stand-off detectors are under development but will not be fielded to a significant degree until FY2002. The USAF has no fielded biological agent detection capability other than small quantities of Portal Shield biological detectors. Based on projected MTW requirements, the Services' shortage of biological warfare detection systems could seriously impact the joint forces' ability to survive and sustain combat operation under NBC warfare conditions and fulfill the current requirement for which they must plan.

### 6.3 Marketplace Factors

There are many marketplace factors that come into play in this technology arena, especially for small size companies who are preparing to go public. Based on interviews with company spokespersons and reviewing Securities and Exchange Commission documentation, the study team compiled a list of marketplace factors, which are synopsized in the following paragraphs.

#### 6.3.1 Marketplace Demand

Currently, there is not enough demand for any single biological detection system that would be the basis for companies to make a realistic business case decision on production. Any commercial system developed must be dual use because the military is (and will probably always remain) too small a segment of the market. It is unlikely that any company would set up a production line for fifty detectors unless DoD/DND paid the development and set up costs. Even if a government paid these costs, it could probably only entice a contractor to set up a production facility if that contractor thought that even greater sales were possible to first responders, foreign military organizations, other civil agencies, etc.

As noted previously, a lot of these companies are not developing technologies solely for use in biological detection systems but rather technologies that could be used in a variety of both defense and commercial applications. The key to their success depends on their ability to successfully commercialize a broad range of products based on the technologies they have developed. Company representatives indicated that if their products do not gain market acceptance across their broad intended range of applications in the various target markets, their business will be significantly harmed. Some noted that their companies have only recently commercially launched many of their current products for sale to these markets, and many of their products have achieved only limited sales. The commercial success of their company depends on obtaining continued and expanding market acceptance of their products. With the significantly stepped up attention to this area from domestic preparedness programs, the companies perceive more marketplace potential for biological detection systems, making this a more attractive area on which to focus.

### 6.3.2 Rapid Technological Change

Another marketplace consideration for companies involved in biological detection technology development is that their products compete in markets that are subject to rapid technological change and frequent new product introductions. Rapidly changing technology could make some or all of their product lines obsolete unless they are able to continually improve existing products and develop new products. If a company's products are substantially based on one specific technology, i.e. mass spectrometry, they are particularly vulnerable to any technological advances that would make that technology obsolete.

In addition, many of the companies' product lines are based on complex technologies that are subject to rapid change as new technologies are developed and introduced in the marketplace. They may have difficulty keeping abreast of the rapid changes affecting each of the different markets they serve or intend to serve. If they fail to develop and introduce products in a timely manner in response to changing technology, market demands or the requirements of their customers, this failure could harm their business.

### 6.3.3 Overhead Project Costs

Many companies offer or plan to offer a broad product line and have incurred and expect to continue to incur substantial expenses for development of new products and enhanced versions of their existing products. Receiving government R&D funding is a great help in this regard. But, several company officials noted that the overhead cost of running small government R&D projects sometimes prevents them from taking on other projects. Some companies stated that they try not to take government projects under $1M. And, after expending time and effort on developing a proposal, there is no guarantee that the company will be awarded a contract. Canadian officials noted that Canadian contracting regulations make it difficult to sole source a contract despite a clearly demonstrated ability to succeed with a technology. Contracts are most often awarded on the basis of lowest cost and the developers of leading technologies may not be awarded the follow-on contracts. Also, the speed of technological change in their targeted markets may prevent them from being able to successfully market some or all of their products for the length of time required to recover their development costs.

Many cited lengthy product development and contract negotiation periods and certain risks inherent in long-term government contracts. These long-term contracts can involve lengthy pre-contract negotiations and product development. They may be required to devote substantial working capital and other resources prior to obtaining product orders. As a result, some companies incur substantial costs before they achieve revenue from these products. Moreover, in return for larger, longer term contracts, several spokespersons said that their customers for these products often demand more stringent acceptance criteria. These criteria also delay a company's ability to obtain revenue from sales of these products. Furthermore, they may not be able to accurately predict in advance their costs to fulfill their obligations under these long-term contracts. If they fail to accurately predict their costs, due to inflation or other factors, this failure may harm their revenue.

### 6.3.4 Technological Competition

Company spokespersons noted that they face stiff competition in each market. For each of their product lines, they face competition from major competitors, including competitors who also offer products based on identical technologies. This is especially true in the life science markets, where many forecast the competition will increase significantly as more biotechnology and pharmaceutical companies adopt automated high-throughput bioanalytical instruments as tools for drug discovery, drug development, proteomics, genomics and metabolomics. The market reality is that their competitors could develop or market products that are more effective or commercially attractive than a company's current or future products or that may render their products obsolete. And, some of these competitors have substantially greater financial, operational, marketing and technical resources.

### 6.3.5 Intellectual Property Concerns

#### 6.3.5.1 Obtaining Patent Protection

Many stated that their continued success will depend in significant part on their ability to obtain and maintain meaningful patent protection for their products throughout the world.

Companies rely on patents to protect a significant part of their intellectual property and to enhance their competitive position. However, a company's presently pending or future patent applications may not issue as patents, and any patent previously issued to the company may be challenged, invalidated, held unenforceable or circumvented. Furthermore, the claims in patents which have been issued or which may be issued to the company in the future may not be sufficiently broad to prevent third parties from producing competing products similar to their products. In addition, the laws of various foreign countries in which the company competes may not protect their intellectual property to the same extent as do the laws of the United States or Canada. Failure to obtain adequate patent protection for their proprietary technology could materially impair the company's ability to be commercially competitive.

#### *6.3.5.2 Avoiding Patent Infringement*

A lot of the companies stated that their success depends on their ability to operate without infringing or misappropriating the proprietary rights of others. Their commercial success depends on avoiding the infringement of other parties' valid patents and proprietary rights as well as the breach of any licenses relating to their technologies and products. There are various patents that may relate to a competitor's technology. A company may be found in the future to infringe these or other patents or proprietary rights of third parties, either with products they are currently marketing or developing or with new products which they may develop in the future. With so many companies involved in developing similar technologies for the same application and competing with each other for market share, this will in all likelihood continue to increase. A number of the companies that the study team interviewed were involved in patent litigation.

If a third party holding rights under a patent successfully asserts an infringement claim with respect to any of a company's current or future products, the company may be prevented from manufacturing or marketing their infringing product in the country or countries covered by the patent they infringe, unless the company can obtain a license from the patent holder. The company may not be able to obtain such a license on commercially reasonable terms, if at all, especially if the patent holder is a competitor. In addition, even if they can obtain such a license, it may be non-exclusive, which will permit others to practice the same technology licensed to the company. The company also may be required to pay substantial damages to the patent holder. It was pointed out that under certain circumstances in the United States, these damages may include damages equal to triple the actual damages experienced by the patent holder. If the company has supplied infringing products to third parties for marketing by them or licensed third parties to manufacture, use or market infringing products, the company may be obligated to indemnify these third parties for any damages they are required to pay to the patent holder and for any losses the third parties may sustain themselves as the result of lost sales or license payments they are required to make to the patent holder. Any successful infringement action brought against a company also may adversely affect marketing of the infringing product in other markets not covered by the infringement action, as well as the company's marketing of other products based on similar technology.

The company in question could suffer consequences of a successful infringement action against them even if the action is subsequently reversed on appeal, nullified through another action, or resolved by settlement with the patent holder. The damages or other remedies awarded could be significant. And fighting such litigation is time consuming and expensive. As a result, any successful infringement action against a company may harm their business and even the threat of infringement action could cause irreparable harm.

#### *6.3.5.3 Loss of Proprietary Information*

In order to protect or enforce their patent rights, many companies may initiate patent litigation against third parties. They also may become subject to interference proceedings conducted in the patent and trademark offices of various countries to determine the priority of inventions. The defense and prosecution, if necessary, of intellectual property suits, interference proceedings and related legal and administrative proceedings is, again, costly and time consuming. Coupled with this is that the company may not prevail in any of these suits. An adverse determination of any litigation or defense proceedings could put the company's patents at risk of being invalidated or interpreted

narrowly and could put their patent applications at risk of not issuing.

Because of the substantial amount of discovery required in connection with intellectual property litigation, there is a risk that some of a company's confidential information could be compromised by disclosure during this type of litigation. If a company is unable to effectively protect their intellectual property, third parties may use their technology, which would impair the company's ability to compete in key markets.

In addition to patent protection, companies also rely on protection of trade secrets, know-how and confidential and proprietary information. To maintain the confidentiality of trade secrets and proprietary information, many companies enter into confidentiality agreements with their employees, consultants and strategic partners upon the commencement of a relationship with the company. However, companies may not obtain these agreements in all circumstances. In the event of unauthorized use or disclosure of this information, these agreements, even if obtained, may not provide meaningful protection for a company's trade secrets or other confidential information. Adequate remedies may not exist in the event of unauthorized use or disclosure of this information. The loss or exposure of their trade secrets and other proprietary information could affect a company's competitive stance. Also, others may have, or may in the future independently develop, substantially similar or superior know-how and technology.

### *6.3.6 Liability Claim Risk*

Companies' manufacture and sale of products could lead to product liability claims for which they could have substantial liability. The manufacture and sale of a company's products exposes the company to product liability claims if any of their products cause injury or are found otherwise unsuitable during manufacturing, marketing, sale or customer use. A successful product liability claim brought against a company in excess of, or outside the coverage of, the company's insurance coverage could detrimentally effect the company's bottom line.

### *6.3.7 Collaboration Risk*

A company's business could be harmed if their collaborations fail to advance the company's product development. For instance, in some cases demand for some companies' products is dependent in part upon the extent to which their collaborations with pharmaceutical and biotechnology companies are successful in developing, or helping these companies to develop new products and new applications for their existing products. In addition, these companies collaborate with academic institutions on product development. They have limited or no control over the resources that any collaborator may devote to a company's products.

### *6.3.8 Strategic Partner Risk*

Many companies rely on strategic partners to market some of their products. For some companies, a substantial portion of their sales of selected products are sales to third parties who incorporate the company's products in their systems. These third parties are responsible for the marketing and sales of their systems. A company has little or no control over the third parties' marketing and sales activities or how they use their resources. Companies' present or future strategic partners may or may not purchase sufficient quantities of products from the company or perform appropriate marketing and sales activities. These failures by their present or future strategic partners, or the company's inability to maintain or enter into new arrangements with strategic partners for product distribution, could materially harm the growth of the business and the company's ability to generate sufficient revenue.

### *6.3.9 Dependence on Customer's Capital Spending*

Any reduction in the capital resources or government funding of their customers could reduce a company's sales and harm its business. Many companies are dependent on capital purchases by their customers. The spending policies of their customers could have a significant effect on the demand for the company's products. These policies are based on a wide variety of factors, including the resources available to make purchases, the spending priorities among various types of equipment, policies regarding spending during periods of decline and changes in the political climate. Any changes in capital spending or changes in the capital budgets of their customers could significantly reduce demand for a company's

products. The capital resources of their biotechnology and other corporate customers may be limited by the availability of equity or debt financing. Any significant decline in research and development expenditures could harm their business.

Some companies are heavily dependent, both directly and indirectly, upon general health care spending patterns, particularly in the research and development budgets of the pharmaceutical and biotechnology industries, as well as upon the financial condition of various governments and government agencies. Companies in this arena benefit from governmental contracts and research grants. Whether they will continue to be able to attract these grants depends not only on the quality of their products, but also on general spending patterns of public institutions. There exists the risk of a potential program change in governmental spending allocations to scientific and medical research fields which could substantially reduce or even eliminate these grants. In addition, a company's sales to non-profit and government entities could be affected depending on how dependent these researchers are on government support for scientific research. Any decline in this support could harm their business.

#### *6.3.10 International Sales Risk*

International sales and operations are and will remain subject to a number of additional risks not typically present in domestic operations, including:

- changes in regulatory requirements;
- the imposition of government controls;
- political and economic instability or conflicts;
- costs and risks of deploying systems in foreign countries;
- limited intellectual property rights; and
- the burden of complying with a wide variety of complex foreign laws and treaties.

A company's international operations are subject to the risks associated with the imposition of legislation and regulation relating to the import or export of high technology products. They cannot predict whether tariffs or restrictions upon the importation or exportation of their products will be implemented by the United States, Canada, or other countries. If these tariffs or restrictions are imposed, the company's revenues or profits could suffer.

#### *6.3.11 Hazardous Material Risk*

Several companies use controlled hazardous and radioactive materials in their business. If an accident with these substances occurs, they could be held liable for any damages that result. Additionally, an accident could damage their research and manufacturing facilities, resulting in delays and increased costs. And, responding to claims relating to improper handling, storage or disposal of hazardous chemicals and radioactive and biological materials which a company uses could be time consuming and costly.

### *6.4 Technology Integration*

In the course of this study, the team had an opportunity to interface with a number of different company representatives. One concern that the team wanted to address was the industrial capacity to manufacture a significant quantity of detection systems. Many of these companies are in the development stages of technological maturity, with very small scale manufacturing capabilities. Coupled with this is the fact that several different technologies are needed as components for a major detection system. Depending on the military's demand for the detection systems, the ability of the industrial base to meet the quantities required was a key factor. What the team discovered is that the companies were well aware of this fact, and that they recognized the necessity to formulate teaming arrangements in order to meet requirements. Many in the community already had such arrangements in place, or were in the process of formalizing such ties. Small companies were teaming with larger companies, who would act as system integrators, purchasing the different components from the various vendors and assembling the entire detection system. Several of the larger scale manufacturers had flexible manufacturing lines, so that if the need for a certain system was of a more immediate priority, the manufacturer could accommodate the rise in demand. Hence, the concern about whether the industrial base would be able to meet the military's demand for such systems was waylaid.

### *6.5 DoD/DND Role in Enhancing Technology Industrial Base*

Stabilized funding is pivotal to the continued involvement of industry in government detection program technologies. A good rapport with industry cannot be established if funding needed for a multi-year program is subject to fluctuations. Industry makes business decisions based on the total level of funding budgeted for that program. On the U.S. side especially, funding provided for a program that has suddenly been stripped has led to the disruption of ongoing industrial programs and caused friction with industry partners.

Sustained, stable funding is key to the success of realizing technological gains in research areas and commercializing these technologies. Fluctuations in funding that small businesses were counting on can be detrimental to that company's business forecast and may disenchant them from doing further business with the government.

Effective communication is vital. Knowing the types of collaborative opportunities that the two governments are seeking helps industry to understand what role they could play and where their technologies might be successfully implemented. The DoD APBI provides a good mechanism for sharing future requirements with industry.

Fora such as the First Joint Conference on Point Detection for Chemical and Biological Defense held in October, 2000 and the recent DRES Chemical and Biological Industry Day provide an invaluable opportunity for the CB community to share ideas, discuss potential technological advances, and collaborate on possible joint opportunities. Conferences of this nature could help to foster improved dialogue between companies, laboratories, and academia possessing the different pieces of a biological agent detection system as well as with the military organizations.

## *7.0 U.S. AND CANADIAN GOVERNMENT BIOLOGICAL DETECTION TECHNOLOGIES RESEARCH AND DEVELOPMENT INITIATIVES*

Canada's research arm for biological detection is centralized at DRES. The U.S. research efforts are more decentralized, more complex, and broader ranging. Many different research components of the U.S. government are involved in U.S. biological detection R&D. Research in this area is conducted by U.S. Service laboratories, as well as within DOE and DARPA. Four of the U.S. federal programs that fund R&D of CB detection technologies covered in this report are:

- DoD's CB Defense Program
- DARPA's BW Defense Program
- DOE's CB Nonproliferation Program, and
- Counterterror Technical Support Program conducted by the Technical Support Working Group (TSWG).

These programs pursue R&D ranging from applied research to prototype development. DoD and DARPA programs concentrate primarily on fulfilling the military's requirements; DOE and TSWG programs are aimed primarily at fulfilling civilian response needs for terrorist incidents. The funding for DARPA and DOE R&D programs has been increasing and combined are projected to be greater than the non-medical R&D funding for DoD's CB Defense Program.

Biological detection technologies may originate from both within the U.S. and Canadian governments and from the commercial sector. Main sources within the two governments involved in researching BW detection systems are:

- DARPA
- The U.S. Services' research and engineering centers (e.g., the U.S. Army's Edgewood Chemical-Biological Center (ECBC), the Naval Research Lab, the Naval Medical Research Center (NMRC), the Naval Surface Warfare Center, and the USAF Research Laboratory)
- The National Laboratories (e.g., Los Alamos National Laboratory, Lawrence Livermore National Laboratory, Lawrence Berkeley National Laboratory, and Sandia National Laboratory )
- DRES.

Biological detection researchers from the commercial sector include:

- Universities and university associated laboratories (e.g., Lincoln Laboratories at Massachusetts Institute of Technology and the Advanced Physics Laboratories at Johns Hopkins University)
- Private and commercial organizations
- Foreign government and non-government groups (e.g., Porton Down, in the United Kingdom, and Bruker-Franzen in Germany).

A large part of the U.S. funding for biodetection devices comes from the U.S. Government, with less than 20 percent from commercial ventures. The U.S. government spends more money than the Canadian government to fund a number of different research programs and system development initiatives in the biological detection area. This is a reflection of the size difference between the U.S. and Canadian defense R&D budgets. The small Canadian R&D budget for CB detection development limits the programs in which DRES can participate and complete. Given funding constraints, considerable progress has been made in technology development. Their entire budget is $C2.4M, which is small given their mandate to protect the Canadian Forces against CB warfare agents.

The U.S. funding process is very involved and lengthy, and sometimes hampers the military's ability to move forward with a promising technology or fund a new program. The U.S. players must defend their programs through the Program, Planning, and Budgeting System (PPBS) process every year. This can cause fluctuations in funding of programs. DND has a shorter, more streamlined decision process in which very few decision-makers are involved and, as such, its funding is much more stabilized.

More emphasis and sustained, stable funding is needed over a period of time long enough to allow the DoD and DND to research new technologies, move technologies out of the R&D base, ensure effective command and control communications with other systems, and field them. Heightened focus and research dollars should be devoted to the biological detection program. There is a clear need for new

technologies, especially with the demanding requirements of biological agent detection and identification. Traditional hardware systems and/or immuno-assay approaches may be less effective in dealing with complex environments such as cities and populated areas. The use of technologies like state-of-the-art power systems, collection systems, and communications and information technology programs for integration into warning and reporting networks need greater investment. This would allow systems to be reduced in size, be more fully automated and ensure interoperability requirements are met. Incorporation of these supporting technologies into new/advanced platforms could allow for the use of robotics, unattended ground sensors, and unmanned aerial vehicles.

### *7.1 Research and Development Goals*

Most detection devices are in either the field testing stage or are still in the laboratory. The development of BW agent detection and identification systems is one of the most intense research activities in defense R&D. The key aim of detection research is to provide a real-time capability to detect, identify, characterize, locate and warn against all known or validated BW agent threats below specified threshold effects levels. The initial focus of current research in the area of biological detection systems is on collectors, generic detectors, early warning technologies and reagents. A number of advances have been made in the last ten years in the areas of BW agent detection methods, alarming algorithms, and identification technologies. In the early 1990s, detection systems were rapidly developed to address the critical need for BW agent detection. These are now being replaced by second and third generation systems incorporating technology strides achieved in bio-aerosol detection, i.e., replacement of flow cytometry with fluorescence particle sizer technology in the JBPDS and automating manual processes. Existing technologies are being enhanced while alternative detection technologies are being developed. BW detection R&D revolves around the ongoing R&D into new technologies for biological detection, including supporting critical reagents, while fine-tuning existing technologies, such as developing improved or novel bio-aerosol detection methods, critical reagents for BW agent identification, and complimentary technologies to immunoassays for identification.

Technologies have become increasingly specific and capable of discriminating natural or ambient bio-aerosols from man-made aerosols to actual BW attacks. Early systems were heavily reliant on manpower to operate what was essentially commercial off the shelf (COTS) laboratory analytical equipment in the field. Systems are now becoming more automated and more autonomous.

The main research emphasis is on the following objectives:

- Improve biological detection sensitivity and identification capability, especially in stand-off and early warning detection, ideally moving towards detect-to-warn capability
- Improve agent discrimination and quantification
- Reduce false positives
- Place emphasis on reduced weight, miniaturization, automation, and field-portability
- Integrate components into a single, rugged system that optimizes power while retaining modularity to support upgrades
- Improve interconnectivity and reduce logistical support required for detectors
- Reduce power requirements
- Fuze sensor data with other battlefield data to display near real-time images of events
- Protect high value, fixed assets such as a field hospital or airfield
- Address affordability.

As documented in a number of research publications, some of the desired features researchers hope to achieve in the design of various future biological detection systems include:

- operable with minimal supporting infrastructure
- operable in a variety of terrain
- must interface with existing and planned command and control systems
- collection equipment and procedures that can handle air, soil, liquid, and surface samples
- robust equipment that can withstand vehicle transport and environmental extremes
- man-portable
- high-volume automated throughput
- inexpensive
- disposable or decontamination-capable

- minimal requirement for specialized training
- operable for long periods of time with minimal maintenance
- long shelf-life
- broad-ranged and able to add new threat agents rapidly
- sensitive to civilian population susceptibility
- low false positive alarm rates that reflect specific mission requirements
- zero false negatives
- rapid detection and identification.

Automation and the minimum use of sensitive reagents are key. The ability to "deploy and leave" the system to operate without user intervention for long periods is highly desirable. This requires that the system operation use a minimal amount of sensitive reagents and that the system's health and performance can be monitored from a remote command post. Detection systems also must be highly mobile to support surveillance of main supply routes and for operation aboard ships.

The two governments insist that the systems must have low false-positive rates. However, some Canadian Government officials noted that there is an expectation that biological detection equipment will detect 100% of events with 0% casualties. They stated that personnel are not willing to compromise on specifications or acquisition strategies. This runs the risk of never fielding a system because it might only work 60% (or 80% or 99%) of the time, but even that is better than what the Services have today. These officials stated that, though this is the ultimate goal to strive for, this is a very onerous requirement based on current technologies and no other weapon system is expected to have this type of success rate – to work 100% with 0% casualties.

Government personnel have stated that they want future biological detection systems that provide rapid detection, confident identification (for down-wind hazard prediction and post attack treatment), and be interoperable with command information management systems like JWARN. It is vitally important that information be shared between air-defense systems and biological defense systems. The ability to achieve rapid warning is the key to minimizing casualties. This entails that, in addition to real time detection, rapid dissemination of the alarm and message to the appropriate levels of the Command structure is required. This would require automating the alarming and messaging functions, and integrating them with the Command and Control system. Thus, the deployment of detection systems must be fully integrated with the other systems being used.

Though the perceived major threat is aerosol delivered BW attacks, Government officials have cited that the ability to detect BW agents in water supplies also is needed. At many military fixed sites, troops draw potable water supplies from uncontrolled civilian sources. Given appropriate instruments for concentrating particulates in water supplies, they believe that current detection and identification technologies should be adaptable to the role of at least semi-automated monitoring of water.

Considered important to reducing the size and the weight of the system, reducing supportability requirements and increasing system utility, is to reduce the power components and develop more efficient power sources into bio-detection. Government officials noted that using technologies like state-of-the-art power systems, collection systems, and communications and information technology programs for integration into warning and reporting networks would allow systems to be reduced in size and eliminate "man-in-the-loop" complexity. Incorporation of these supporting technologies into new/advanced platforms could allow for the use of robotics, unattended ground sensors, unmanned aerial vehicles, and man-pack.

Another need of future enhancements to current detection systems is to incorporate technologies that enable better characterization and portrayal of background interference for point and stand-off bio sensors.

Government officials also wanted to see systems developed that were capable of non-specific identification, e.g., determining the presence of bacteria, toxins and viruses by targeting generic factors. They perceive that broad based detection may provide a means for detecting biologically engineered threats with signatures that are different from the agents current systems are programmed to identify. Immunoassays have been one of the most effective technologies used for rapid identification of human etiological agents. However, with advances in molecular biology, the capability for designing novel threats undetectable by immunoassay is a reality.

Thus, a genetic identification capability is required to identify threats where immunoassays or other technologies are limited; for example, in the case of genetically engineered threats. Assay based identification requires prior knowledge of the type of BW agent being used and will not identify unknown agents. A genetic-based, complementary identification system could help surmount this issue of detecting unknown agents.

Improved sample collection systems for air, surfaces, water, and soil also are wanted. Government officials noted that current DNA based detection/identification technology (e.g., PCR, MS) requires clean samples for analysis. Sample preparation for DNA analysis is currently a manual, somewhat lengthy process. However, current wet-chemistry techniques are more rapid and reliable when confronted with a dirty sample. DNA based detection/identification is feasible for military field detection requirements only after a sample has been collected, contaminants have been removed from the sample, and a clean sample (inhibitors removed) has been presented to the identification component. The defense departments believe that the speed of detection using DNA-based detectors could be accelerated with the development of improved sample preparation systems.

Other defense requirements that have been cited are:

- hand-held bio-sensors that detect airborne biological agents with clear signal, affordable, and with a low false alarm rate.
- bio-detection devices that can continuously monitor the air for BW agents,
- non-intrusive detection of biological agents (e.g., screening cargo, mail, packages, etc.).

Several stand-alone detectors are being developed to fulfill these requirements. Key factors in achieving these goals are to reduce the dependence on reagents and size, weight and power requirements of existing systems. A number of research programs are in place addressing these technological challenges.

Single detectors functioning alone will not give the broad area coverage required for early warning. Systems and networks of a variety of detector technologies are needed for full coverage. A lot of emphasis has been placed on developing arrayed point biological detection systems integrated into command and control to provide wider area coverage for such fixed assets like a base or a port. But achieving effective stand-off detection capabilities are daunting. According to the DoD *Chemical and Biological Defense Program Annual Report to Congress*, March, 2000, this is due to a number of different factors including: a lack of fundamental data in understanding the spectral properties of BW agents, the range limitations of lasers due to atmospheric absorption, and natural background interference. Two different concepts are being promoted to achieve an effective stand-off capability, but further efforts in this area are not scheduled in the current technology planning cycle until FY2002 and FY2003.

The focus for the future is on developing multi-agent sensors for biological agent detection and remote/early warning CB detection. One challenge facing the community is to ensure the effective integration of new and emerging sensor technologies into current and future detection programs.

Researchers are striving to integrate CB point and remote/early warning detection components into a single system which will be placed into various sea, air, and ground platforms and into automated warning and reporting networks linked with command, control, communication, computer and intelligence networks. The realization that these systems need to be integrated and networked to provide wide area coverage is a driving consideration of the next generation of systems being developed.

Alternative concepts for biological agent detection and active defense should continue to be explored. At present, there is no silver bullet for universal detection of BW agents. No one method or technique exists today that is capable of detecting all agents. Potential alternatives to currently employed technologies, perhaps discovered through technology breakthroughs achieved as a result of research being conducted in other scientific fields, could advance the capabilities of existing systems.

A bottom-up review of future biological detection requirements and operational concepts with emphasis on integration, interoperability, and operational utility would be useful. The current point detection systems all deal with detection of agents after people have been exposed, and the next step is medical rather than

operational. Increased emphasis should be placed on the larger, operationally useable system-of-systems concept that could maintain operational effectiveness in a BW environment.

### *7.2 Technological Challenges*

The potential for creating genetically manipulated or novel BW agents poses a significant challenge for biological detection. To guard against this threat, many researchers are looking at ways to detect agents by using ab initio principles - more generic methods of detection - to determine if a particular biological agent is a hazard independent of any previous knowledge about the threat.

Another technological hurdle involves the gap between the threat aerosol concentration and concentration limits of detection of current equipment. Both detection and identification systems require high volume, high efficiency collectors to sample the air and present a sample to the detector. The weight and power consumption of these collectors is often a major problem in the design of affordable or deployable detectors. Further research into sample collection and processing is required. Some scientists have stated that alternative approaches to sample concentration are being researched and that technological breakthroughs could be achieved in electrostatic filtration and corona discharge.

Further research also is needed to understand the effect of background bio-aerosol. As noted by several researchers, air is a complex biological matrix consisting of naturally occurring and man-made aerosols, which can undermine a detector's ability to discriminate innocuous events from BW attacks. Understanding of the background aerosol's makeup (i.e., bacteria, virus, fungal and proteomic content) and variation (i.e., weather, climate, and geography) is necessary for developing alarming algorithms and information processing in BW detection.

Another research concern cited was the lack of understanding of the principles of BW and how biological detection systems can be effectively employed to protect military personnel in the field. As advances in BW detection systems have been made and these systems have been deployed, there is a training gap with regards to what these new systems can do for actual force protection during operations. The detectors provide data, which in turn needs to be interpreted and evaluated, and then communicated. But without effective training on these systems within the military community, the capabilities of these next generation systems will not be recognized.

As operations become increasingly joint and combined, the need to ensure the compatibility of systems, interchangeability of detectors and interoperability of data and information inputs in command, control and information systems is underscored. This also stresses the need for joint/combined training.

To validate the different technologies under development, JFT are conducted at Dugway Proving Ground, Utah, and DRES in support of biological defense programs within DoD, DND and the United Kingdom. Sponsored by the JPO-BD, the JFT process employs analytical methods based on biological detection system requirements to weigh component characteristics and to assess the maturity of the technologies for inclusion into legacy systems, e.g., LR-BSDS and BIDS, and emerging biological detection systems. Recently, these trials have generated interest from outside the military arena as well. Because of the expansion of charters into biological defense amongst other government agencies, JFTs have become a critical tool for the Federal Bureau of Investigation, first responders and other domestic preparedness agencies. Currently, there is a real backlog in testing of different detection technologies.

A concern that has been cited is that there are insufficient test sites in the U.S. to accommodate all the required testing. In fact, the latest JFT was held at DRES. The JFT process is being standardized between the primary U.S. and Canadian facilities. Standard test methodologies, processes, and procedures are in place based on previous JFT and the tri-national Joint Field Trials Test and Evaluation Working Group efforts (formed under the auspices of the MOU Cooperative Program on Chemical and Biological Defense Materiel and Planning Guide for Commanders – now the MOU Research, Development and Acquisition of Chemical, Biological, and Radiological Defense Materiel (CBR MOU)). This will allow U.S. and Canadian researchers to compare data based on the same reporting results criteria. Additional work must be accomplished in developing and implementing new test methodologies to

appropriately test emerging point and stand-off technologies. Improved standards will also provide U.S. and Canadian researchers with the opportunity to directly compare data from different testing sites and analyze the effectiveness of different technologies in order to gauge what programs and technologies should be targeted for transition. It is conceivable that, in the future, with these guidelines, industry could have their technologies tested at different testing sites and submit their data to the JFT Joint Abbreviated Analysis for analysis.

The JFTs provide an opportunity to objectively evaluate potential technologies for inclusion in BW agent detection systems. These tests provide materiel developers with opportunities to conduct field and chamber testing on their technologies while gaining performance data early on in their programs that they wouldn't otherwise be able to afford. It is an excellent opportunity for them to showcase technologies that have great potential, but lack strong sponsorship. These reports are also open to other appropriate government agencies for their uses. The JFT process has been touted as setting the standards for domestic and international biological detection test methodologies. This process has been adopted by Canada and the UK, and set the baseline for JFT VI which was completed in September, 2000 in Canada.

The JFTs are somewhat of a disadvantage to smaller companies with tight budgets. Participants in JFTs must bear the cost of their own travel, accommodations, and equipment for the duration of the trials. Some promising technologies are being developed by small companies that do not have the internal resources to participate in the JFTs.

### *7.3 R&D Collaboration*

As cited in numerous General Accounting Office (GAO) reports and documented in discussions with government and commercial biological detection system researchers, the U.S. R&D programs conduct R&D in overlapping areas as well as in support of similar user communities. They pursue many of the same capabilities and contract with many of the same laboratories to perform the R&D work. Though there is formal and informal program coordination between the agencies sponsoring R&D, the GAO noted that it is inconsistent and does not ensure that potential overlaps, gaps, and opportunities for collaboration are addressed. They asserted that coordinating mechanisms lack information on prioritized needs, validated CB defense equipment requirements and how programs relate R&D projects to these needs. The requirements process needs to be defined. This lack of defined standards and processes for measuring the technologies to these standards has concerned industry as well.

The DoD biological detector system requirements need to be defined. Competing priorities of a very complex management and oversight bureaucracy can dilute program focus. To help alleviate this, the DoD has developed an organization structure to ensure close and continuous coordination of CB warfare defense. The Services now jointly prepare modernization plans; Research, Development, and Acquisition (RDA) plans; and Joint Logistics Support Plans for NBC defense programs. The DoD intends to submit the needed requirements information to Congress in 2001. The DoD *Chemical and Biological Defense Program Annual Report to Congress*, issued in March, 2000, outlined the mission and goals of the DoD CB Defense Program (CBDP). Working within this framework, the DoD is in the process of developing performance goals and measures, which will be used in the development of the CBDP Strategy Guidance and planning, programming, and budgeting documents. The DoD anticipates that this plan will be completed in 2000 and will be included in the next annual report to Congress.

The same criticism has been leveled at DOE and DARPA sponsored programs, with the GAO citing that these programs do not formally utilize user requirements in planning their R&D goals. The GAO stated that domestic preparedness needs are not as clearly defined and not specified in as great a detail as the military has defined their requirements. No detailed equipment performance specifications or mission and threat analyses documentation has been prepared. In a 1999 GAO report, the GAO stated that, since 1996, the CB program has experienced rapid program growth without development of a government-wide strategy that includes a defined end-state, soundly established, defined and prioritized program requirements, and crosscutting analyses of individual agencies' budget proposals to ensure that unnecessary duplication and waste are avoided and existing federal, state and local capabilities are fully

leveraged.[45] Though OMB has performed two government wide reviews (in 1998 and 1999) of funding levels and programs to combat terrorism that provide insight into enacted funding and budget requests, the reports do not clearly or explicitly describe any established priorities or duplication of efforts as called for in the legislation. The GAO noted that rapid growth is taking place in the domestic preparedness programs for responding to terrorist attacks and public health initiatives, though no sound threat and risk assessments to establish program requirements and prioritize and focus the U.S. investments has been accomplished. In the domestic preparedness arena, many similar programs and initiatives across several agencies, such as those in fire, police, and emergency medical services, to deal with the consequences of a terrorist attack have been instituted, lending to potential overlap and redundant efforts.

DND has had a smiliar experience on this same point. The DND is maturing a concept of operations specifically for biological agent detectors that the R&D community can use to move forward. However, some Canadian government officials stated that the DND needs to express a clear need, provide the focus for development activity and provide the stable funding for the necessary research efforts.

The most effective coordination in U.S. CB defense efforts appears to be among the U.S. Military Services, who work jointly together through the CB Defense Program, which has led to a number of joint Service projects. This program is geared to developing a joint Service approach to CB defense RDA and is aimed at eliminating unnecessary redundancies and leveraging common technologies and requirements. Led by the Joint Service Materiel Group (JSMG) through the Contamination Avoidance Commodity Area Manager, RDA efforts were undertaken which share common technical goals. Through this process, the Services have been able to maximize limited resources and focus on joint, high priority needs while reducing the number of different end items, manpower and logistics needed to support this equipment.

The JPO-BD and the Joint NBC Defense Board are active and effective in coordinating joint Service requirements. In the last few years, 44 separate Service contamination avoidance developmental efforts were consolidated into ten fully coordinated joint projects.

A reality that must be considered in developing joint programs, however, is that each of the Services also has unique, specific requirements for biological detection systems to meet their needs. According to a National Defense article, meeting the needs of all Services using common equipment is difficult, hampering the effectiveness of joint programs.[46] For instance, whereas the USAF can handle a 900-pound detector, the USMC want a detector that weighs just nine pounds.

A key hurdle in effective coordination is determining how best to collaborate between civilian and military CB detection measures. There are different decision-makers involved in determining military and domestic response issues. How to coordinate requirements and program initiatives between these communities and determine what role the DoD and DND should play in civilian biological defense needs is a real challenge. Considering that the funding for DARPA's and DOE's R&D programs has been increasing and, combined, are projected to be greater than the non-medical R&D funding for DoD's CBDP for FY 2001, mechanisms for coordination need to be established to ensure that funding is used most effectively, redundant efforts are avoided, and similar requirements are handled jointly. A formal process to coordinate areas of research that are supported by multiple agencies and nations at a senior government level could aid this coordination process by providing a mechanism to share insights on technology advances/drawbacks, and enhance opportunities for collaboration.

Closer R&D cooperation between DoD, DOE and civil biological defense first responders would foster ties that would also improve coordination during responses to domestic biological incidents. In the event of a domestic incident on American soil resulting in the release of chemical, biological, radiological or nuclear materials or high-yield conventional explosives, the local law enforcement, fire and emergency medical personnel who are first to respond may become rapidly overwhelmed by the magnitude and lingering effects. In that instance, a governor may request a presidential disaster declaration for the state and assistance from the federal government through the lead federal agency. If DoD assistance is requested, the DoD

has many unique capabilities, both technical and operational, which could support civil authorities to mitigate and manage the consequences of such an incident. DoD would provide support to the lead federal agency – Federal Emergency Management Agency for domestic consequence management operations or the Department of State for foreign consequence management operations.

As detailed in the January, 2001 OSD report "Proliferation: Threat and Response", DoD has established 27 WMD Civil Support Teams (CSTs), composed of 22 well-trained and equipped full-time National Guard personnel. Upon completion of training and certification in FY 2001, one WMD CST will be stationed in each of the ten Federal Emergency Management Agency regions around the country, ready to provide support when directed by their respective governors. Their mission will be to deploy rapidly, assist local first responders in determining the precise nature of an incident, provide expert medical and technical advice, and help pave the way for the identification and arrival of follow-on military support. Unless federalized, the CSTs will remain state National Guard assets that can be quickly accessed by proximate governors. By congressional direction, DoD is also training 17 additional WMD CSTs whose certification is anticipated in FY 2002. Congress authorized an additional five teams to be established in FY 2001. Their training and certification is also anticipated in FY 2002.

The DoD understands the challenges they face in biological detection and have set forth precise performance requirements, coupled with clear, matching R&D objectives to fulfill these requirements. How the JPO-BD designed the JBPDS Block 1 Program illustrates this rather rigorous planning process. According to a paper prepared by Battelle Memorial Institute, during the first few months of the project, a trade-off analysis was performed to identify the technologies that could best meet the performance requirements (85 in all). This began with the identification of over 60 technologies that were downselected to approximately 20 technologies that were deemed potentially viable for the JBPDS. The candidate technologies are shown in the following table. Virtually all of these technologies initially had been evaluated during the JFTs.

**Table 7.1, JBPDS Initial Candidate Components**

| TRIGGERS | COLLECTORS | DETECTORS | IDENTIFIERS |
|---|---|---|---|
| Ultraviolet Aerodynamic Particle Sizer (UV APS) | Carousel Liquid Sampler (CRLS) | FLuorescence Aerodynamic Particle Sizer (FLAPS) | Chemical/Biological Mass Spectrometer (CBMS) |
| FLuorescence Aerodynamic Particle Sizer (FLAPS) | IBADS Wetted-Wall Cyclone Sampler | Aerosol Shape Analysis System (ASAS) | Biological Detection Kit (BDK) |
| High Volume Aerodynamic Particle Sizer (HVAPS & HVAPS II) | Spincon | Biological Aerosol Warning System (BAWS) | Hand-Held Assays |
| Interim Biological Agent Detection System (IBADS) APS | Biological Inertial Collector/Concentrator (BICC) | Single Particle Fluorescent counter (SPFC) | Light Addressable Potentiometric Sensor (LAPS) |
| Aerosol Shape Analysis System (ASAS) | Research International 1, 2 Collectors | Surface Transverse Wave (STW)/Shear Horizontal-Acoustic Plate Model | IGEN Origen |
| Met One Particle Counter | Phtlaas | Chemical/Biological Mass Spectrometer (CBMS) | Fiber Optic Wave Guide (FOWG) |
| BIO-TEST Particle Measuring System | RECON test system PM-10 | Model 4700 Bio-Luminometer | Biological Detector (BD) |
| Aerosizer | French Cyclone | Model 3550 Bio-Luminometer | Mini-PCR |
| | | IDEXX Lightning Bio-Luminometers | Enzyme Linked Immuno Sorbant Assay (ELISA) |
| | | Coulter Flow Cytometers | |
| | | LANL Flow Cytometer | |
| | | Becton-Dickenson Flow Cytometers | |
| | | Bio-Rad BRYTE Flow-Cytometer | |
| | | Lawrence Livermore National Laboratory Flow Cytometer | |

These remaining technologies were subsequently combined into 99 systems that were evaluated using an Analytical Hierarchy Process. Considerations included: performance, size, weight, power consumption, life cycle costs, level of automation, and developmental maturity. The participants have not made a decision on the final technologies as of December 1, 2000.

There are a lot of new players in the biological defense arena, and improvements in communication are needed. Though there is formal and informal program coordination between the agencies sponsoring R&D, it is inconsistent and does not ensure that potential overlaps, gaps, and opportunities for collaboration are addressed. In a JPO-BD briefing, they cited three challenges:

- One challenge facing this community is the ability to leverage mission requirements for Domestic, Reserve, and National Guard requirements
- Another challenge is to overcome the instability of Service requirements.
- A third challenge is to instill international collaboration.

In an effort to accomplish just this, the First Joint Conference on Point Detection for Chemical and Biological Defense was sponsored on October 23-27, 2000, by the Joint Science and Technology Panel On CB Defense (JSTPCBD) in cooperation with the U.S. Army, U.S. Navy, USAF, USMC, and other CB agencies. The conference addressed issues related to development of point detectors based on state-of-the-art methodologies for reconnaissance, detection, identification, and quantification, of CB agents. Particular attention was given to the subsystems that make up a CB Point Detector. Sessions were also held addressing technologies that address Joint User needs in the areas of:

- identifying non-standard CB agents and Toxic Industrial Materials (TIM);
- minimizing false positives in the detection of CB agents;
- increased detection sensitivity and reduced detection response times and;
- integration of CB detection capabilities.

Additionally, the conference focused on test issues, new technology concepts and future needs. The overall objective of the conference was to bring together experts and practitioners for a review of the state-of-the-art science and technology of CB point detection and related technologies to serve as the basis for the formation of a comprehensive DOD program strategy for the development of the next-generation CB point detection systems.

The DoD and DND participate in a number of international cooperative and collaborative CB efforts to leverage technology development and achieve commonality, interoperability, and system integration among allies and coalition partners. According to the DoD *Chemical and Biological Defense Program Annual Report to Congress*, March, 2000, the U.S. has in place fifty Data Exchange Agreements with fifteen countries, two Technology Development Project Agreements, one MOU, and over 100 scientists and engineers participating in exchange programs on CB issues. Canada, the United Kingdom, and the U.S. have effectively utilized the CBR MOU to foster coordinated and more focused activity in the area of biological detection and should continue to use this agreement. In 1991, the countries realigned their biological detector programs through this mechanism to tackle shared problems and technological hurdles. Because of this agreement, the JFT Test and Evaluation Working Group was established, which resulted in the establishment of JFTs. The countries also have shared information on their respective critical reagents program as a result of this MOU.

An active working group formed as a result of the CBR MOU is the Biological Detection Working Group, whose goal is to pursue cooperative/collaborative efforts in the areas of doctrine, training, CONOPS, and material development. This implies building confidence in the systems used by the other nations and understanding/accepting the significance of an alarm – especially from another MOU nation's sensors/detectors.

Another effective coordinating mechanism has been The Technical Cooperation Program (TTCP), Chemical, Biological and Radiological Defence Group Panel 10 – Detection of Biological Warfare Agents. Through this technical committee, the US, Canada, the United Kingdom, Australia and New Zealand have been active in collaborating on research initiatives of mutual interest.

In addition to these coordinating mechanisms, the U.S. also has three Technology Development Project Agreements in the discussion phase and an additional MOU in negotiation. Technology strides gained as a result of this international cooperation include the ability to detect and identify bacterial spores, enhancements to the downwind hazard model, current detector/monitor technology, and the development of standards for measuring biological backgrounds. However, many U.S. and Canadian government officials believe that the two countries should be more proactive to facilitate cross border research and partnering.

The DoD and DND are experiencing a "brain drain" in the biological detection arena. A number of people who have spent most of their careers studying this area are retiring or have retired. This area of expertise is extremely technical and the loss of such corporate knowledge is disruptive to ongoing research.

### *7.4 DoD Research Efforts*

DoD's program is executed by several organizational elements and coordinated through the Office of the Under Secretary of Defense (Acquisition, Technology and Logistics).

#### *7.4.1 DARPA*

DARPA's mission is to research breakthrough concepts and technologies that could help strengthen the U.S.'s national security. DARPA's BW Defense Program goal is to complement the DoD CB Defense Program by anticipating threats and developing defenses against them. The Agency invests in early technology development phases of programs, and their role decreases in the succeeding stages leading to system development and deployment. Their budget for FY2000 for this program was approximately $132M.

DARPA is developing detection systems which are robust, unattended, and highly sensitive (2-10 particles), as well as biological sensors which are small (< 5 pounds), and low in cost (< $5K). Their focus is on development of technologies with broad applicability against classes of threats. DARPA is seeking to develop and demonstrate detection systems for identifying a broad range of BW agents in the environment, including those that may be encapsulated or bioengineered. DARPA is primarily interested in developing new signatures for detecting and identifying biological agents including spores, vegetative bacteria, viruses, toxins, and bioregulating compounds. Examples of signatures would include peptides, aptamers and phage. These are being sought to serve as adjuncts and/or replacements for antibody identification of bioagents. These new signatures would be inserted into platforms currently under development in order to significantly enhance platform capabilities, to increase sensitivity, specificity, and reliability, and minimize false alarms. DARPA is also seeking methodologies for signatures for rapidly distinguishing between live versus dead bioagents at low concentrations, including samples taken after decontamination procedures have been executed.

DARPA also is looking to develop point-detection technologies that will allow for the rapid identification of suspect agents. The focus of the technologies is directed at biological detection/identification for military use, in battlefield and airbase scenarios, as well as in urban environments. Such detection devices can be small (hand-held) instruments for individual soldier use or automated, dispersed, remotely placed, and networked. DARPA wants to invest in improvements to the current identification technology, that is dependent on time-consuming amplification: for antibodies, the molecular binding event is amplified and for genomic DNA, the DNA molecules themselves are amplified prior to detection. The amplification step adds appreciably to the time and complexity required for the identification of a biological agent. Their goal is improvements to detection technologies to achieve high sensitivity and a low false alarm rate (both false positives and false negatives) with minimal sample pretreatment (including amplification) before detection.

The following enabling technologies are currently under development to support this goal: upconverting phosphors, a phylogenic microchip, the enhancement/replacement of antibodies, and mass spectrometry technologies. Upconverting phosphors are being developed to replace fluorescent reporters. A phylogenic microchip, containing an expanded hierarchical set of more than 100 oligonucleotide probes, is being developed which will enable the parallel detection and identification of a variety of species of organisms allowing rapid determination in unknown samples.

Enhancement/replacement of antibodies is being approached by developing small molecular weight compounds with high affinities and specificities that will enhance current antibody identification protocols, with the ultimate objective of using these kinds of moieties to replace the antibody as the principal detection/identification molecule in biosensors.

The goal of DARPA's Sensor Integration and Modeling for Biological Agent Detection (SIMBAD) program is to develop and demonstrate fully integrated well-characterized sensor system prototypes for CB agent detection. In this context, a sensor system is a complete end-to-end capability of fully integrated technologies capable of monitoring the environment and providing an automated decision output regarding the presence or absence of a threat. Under the SIMBAD program, DARPA has put together multi-disciplinary teams consisting of such disciplines as biology, chemistry, medicine, mathematics, physics, fluid dynamics, computational science, and engineering, including systems engineering to develop and optimize the performance of current and emerging sensor technologies in an attempt to produce systems that far exceed the current state of the art for CW and BW sensors. BW agent sensor systems are the primary goal, with CW agent sensor systems a secondary goal. The ultimate product of SIMBAD is one or more fully integrated and well-characterized sensor systems capable of responding to the threats defined during the duration of the SIMBAD program.

SIMBAD includes, but is not limited to, the following technologies:

- Time-of-Flight Mass-Spectrometers
- Antibody based sensors
- PCR-based sensor for DNA analysis
- Hyperspectral imaging micro-Raman biochip sensor module
- Biofluorescence LIDAR for triggers and stand-off detection of bioagents
- Micromachined aerosol collectors
- Sensor network architectures.

#### *7.4.1.1.1 DARPA Biological Detection Technology Development Initiatives*

Some of the ongoing DARPA programs in the biological detection technology arena are highlighted in further detail in the following subsections.

##### *7.4.1.1.1.1 Analytical Methods Development and Mass Spectrometer Library*

One of DARPA's initiatives, Analytical Methods Development and Mass Spectrometer Library, is aimed at creating more efficient and effective miniature sampling devices that concentrate contaminated air and enhance the ability to capture BW agents. This R&D effort is geared to designing small, high affinity molecules to bind specific biological agents, replacing antibodies that are currently used in detection systems.

In order to detect that the binding of an agent has occurred, the binding event must be magnified. Traditionally, this has been accomplished by tagging the antibody molecule with a fluorescent probe. This program will replace fluorescent tags with upconverting phosphors, will minimize the size of the sample required, and will determine pathogenicity and viability of the agents. The need for the use of fluids in the detection of biological agents also will be eliminated by using a miniaturized (about the size of a shoebox) time-of-flight mass spectrometer.

DARPA has contracted with the Johns Hopkins University Applied Physics Laboratory (JHU/APL), the University of Maryland Baltimore County (UMBC), and the Johns Hopkins University School of Medicine to design and execute signature measurements of threat simulants by MS, a technology that DARPA believes may offer the most robust capability for speed, signature bandwidth, and specificity. Their research will develop the analytical chemotaxonomic methods and novel Time-of-Flight devices used to gain access to the biomarkers, and will also develop the library of signatures against which new spectrometer field measurements will be compared. Simulants such as *E coli*, B-subtillis, E herbicola, and MS-2 capsid protein will be characterized using single and tandem mass spectrometer systems and soft ionization techniques.

The biomarkers and signature libraries that are developed will be used with new mini-mass spectrometers. Through these efforts, DARPA hopes to assure the diversity of the investigation for in-depth characterization and exploitation of the bacteria, viruses, and proteins of interest.

The signature library that DARPA establishes is intended to be used in defense and counterproliferation field measurements.

#### *7.4.1.1.2 Micro Array of Gel-Immobilized Compounds (MAGIChip)*

Another DARPA program involves developing biological microchips for field analysis of microorganisms and toxins. The objective of this program is to develop a biological MAGIChip detection system that can simultaneously identify a vast number of biothreat agents, including bacteria, viruses, fungi, and toxins. Both pathogeneic microorganisms and plasmid-associated toxin genes that might be inserted into otherwise innocuous microorganisms should be detected through this new system. Their goal is to produce a simplified, three-dimensional chip for use in field analysis. The researchers hope to automate the system in the future. Their emphasis is on development of the 3-D chip technology and an automated prototype instrument.

DARPA has partnered with Argonne National Laboratory, Northwestern University, and U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to develop the MAGIChip. These chips are touted as capable of analyzing large amounts of biological information and able to conduct parallel analyses in order to identify genes, proteins, and chemical compounds. The chip is a glass or silicon surface covered by an array of hundreds to thousands of tiny gel pads. Each gel pad represents an individual microchip element, and can contain a specific, chemically immobilized oligonucleotide, DNA, RNA, protein, antibody, receptor molecule, or other compound. They can be used to conduct different chemical and enzymatic reactions. This chip has been shown to effectively detect a single base change among the three billion bases of the human genome.

Applications for biological microchips are growing, including medical diagnostics, monitoring food and drinking water, environmental bioremediation, and crime analysis. The researchers think that this chip detector will ultimately have the ability for rapid, sensitive, specific, and simultaneous detection of many BW agents, including bacteria, viruses, fungi, and toxins.

Through this program, DARPA hopes to accomplish the design of a detector that will provide simple, fast, accurate identification of microbes and toxin genes, with no false alarms. Other perceived benefits include:

- Discrimination among thousands of different immobilized compounds through parallel analysis of thousands of microchip elements
- Rapid, highly sensitive sample identification through fluorescence detection
- Simultaneous thermodynamic measurements on microchip elements, providing thousands of binding constants for various compounds interacting with substances immobilized with the microchip elements
- Flexibility to conduct different chemical and enzymatic procedures on chip elements, so that the MAGIChip can function as a vast array of individual micro-test tubes.

#### *7.4.1.1.3 Biological Agent Detection by Spore Specific Phosphorescence*

This research entails investigating the generation of bacterial spore-specific phosphorescence that would constitute the basis for detecting the viability and quantitation of the bacterial spores of the simulants of biological agents, such as B anthracis and C botulimmi. Through this program, researchers hope to develop a phosphorescence-based sensor integrated with one or more inert matrices suitable for on-site and/or remote sensing of the biological agents in liquid and aerosol modes in the sensitivity range of 100 spores or less. It is based on the projected reaction of a selected sensor component with spore-specific chemical compounds, leading to the formation of a product with characteristic phosphorescence emissions. They believe that this system could be designed and fabricated for a wide range of detection devices, ranging from a simple hand-held system to sophisticated instrument packages. DARPA has teamed with Illinois Institute of Technology Research Institute (IITRI) on this effort.

#### *7.4.1.1.4 Next Generation, Integrated Biosensor Research*

This program hopes to leverage several other DARPA research efforts to create a next generation, integrated biosensor. Key to this effort is the use of a new material on the battlefield, aerogel, which is to be used as a multifunctional material with smart sensing. The

researchers are trying to integrate the smart aerogel into a breadboard prototype biosensor. Aerogel properties of complex pore structure, high internal surface area, and hygroscopicity are being fully utilized to synthesize a smart collection medium that is internally coated with bioaffinity compounds with high specific binding potential to unique pathogens. This will result in isolation of the pathogen by size and type that allow area-limited signal transduction to achieve simultaneous detection and identification. Researchers envision that this new sensor will be able to meet the functional requirements of the next generation biosensor and be lightweight and small for deployment on microsized airborne vehicles.

Through this initiative, researchers believe that they will be able to converge some subprocesses - collection, preparation, and assay - into a unified process using the multifunctional aerogel. Aerogel is a term used to describe very low-density, highly porous, polymeric materials that provide an efficient, lightweight collection medium for airborne particles. They envision that users will have the ability to specifically detect and identify BW agents. The bio-aerosol pathogens will be isolated by type and particle size in specifically engineered smart aerogel docking sites, allowing for parallel detection and identification through future signal transduction and non-specific noise reduction. Other goals for this detection technology are to eliminate fluidics, offer a system that is both lightweight and of micro-size scale, and one that has expandability and bandwidth.

Several research institutions are involved in this effort: Pacific Sierra Research, Inc., University of Virginia, University of Alabama-Birmingham, and the NRL.

#### *7.4.1.1.5 Upconverting Phosphor Compact Handheld Biosensor*

This research is geared towards development of a handheld biosensor that is capable of rapid and sensitive screening of liquid samples for a variety of pathogens and toxins at very low levels with a response time of minutes. Potential applications include battlefield detection of biological weapons and rapid medical diagnosis of disease.

The objective is to develop a new reporter material for biological agent identification and incorporate the technology into a handheld instrument based upon SRI International proprietary upconverting phosphor-diode laser technology. This research uses sub-micron micropheres of upconverting phosphor material as the reporter system, and a single infrared diode laser as the excitation source, allowing for a greater degree of microflexing. The goal is to allow the user to employ this compact handheld biosensor to simultaneously detect two simulant targets, that can be extended to eight or more targets through the use of additional phosphor colors.

This effort also entails reagent development and assay optimization, in conjunction with sampling and detection format optimization for rapid liquid sampling in the field. This work will culminate in the design of a small, self-contained, easy-to-use field sampling chip that can be mass produced and contains all the reagents necessary to conduct sensitive assays.

DARPA will transition the reagents, assays, and instrumentation developed into the commercial marketplace through subcontracts with 3M and a teaming partnership with STC Technologies. 3M will manufacture the disposable fluidics chip samplers, and possibly the handheld biosensor under license to SRI International and STC. STC Technologies is the commercial licensee to SRI International for the upconverting phosphor technology, and will be the commercial supplier of reagents and supporting analytical instrumentation. The technology will be transitioned to U.S. Army Edgewood Research, Development and Engineering Center (ERDEC) and other agencies.

#### *7.4.1.1.6 Structure-Based Ligands to Capture Microorganisms*

DARPA's work in this area is to develop a miniaturized inexpensive device for detection of biological pathogens. Key to this effort is the design of biosensor molecules. Previously, the molecules of choice for biosensoring were antibodies, which are protein molecules generated in mice. However, these molecules have proven unstable in environments where detection systems are designed to be used, with the antibodies losing activity in a dried state. DARPA has teamed with the University of Alabama and IBBEX, Inc. to use structure-based drug design and combinatorial chemistry technologies to design specific, high affinity

small molecule ligands to attach to the surface proteins found on the pathogens. By accomplishing this, they can develop chemically stable ligands with specificity and affinity to meet the requirement of the detection device.

Perceived benefits of this research include:

- Provide high affinity ligands for spores and viruses that will be used as the biosensor molecules in detection devices
- Develop chemically stable molecules that can be used in harsh environments such as dry conditions to replace antibodies
- Provide biosensor molecules which are stable and easily manipulated
- Provide high affinity ligands which could be an alternative for pathogen identification
- Provide ligands which could be used to develop drugs to stop the infection caused by exposure to pathogens.

The University of Alabama team is working with Pacific-Sierra Research, Inc. and Johns Hopkins University Applied Physics Laboratory to develop the prototype detection device. Other scientists involved in DARPA-sponsored projects will be able to integrate these ligands into the detection systems as well.

DARPA will work with a major pharmaceutical company to help transition the drug design technology once clinical trails are initiated.

#### *7.4.1.1.7 Detection of BW Agents*

In this research effort, a team of people with expertise in chemistry, microbiology, electronics, physics, and engineering, are working together to produce a prototype instrument capable of rapid and sensitive detection of BW agents based on capturing the target out of a solution onto a solid surface. However, using solid phase capture can lead to some problems, namely non-specific binding which leads to a low signal to noise ratio, and thus, undesirable detection limits. One goal of this effort is to lower the non-specific binding, which will increase the signal to noise ratio and lead to higher sensitivity. To accomplish this, they are combining unique biomolecules that are sensitive to temperature changes during their binding events with those that are specific for the target of interest. By mixing and matching these molecules in various combinations, the scientists intend to develop a single device which will detect targets in parallel. Another piece of this program is to develop a photometer with a large dynamic range to address the issue of signal transduction, thus allowing users to detect a very wide range of target concentrations. At present, the photometer is designed to detect ten signals simultaneously. Their intention is to improve the photometer to enable it to detect at least 20 targets. Through this R&D thrust, the scientists hope to develop a set of chemistries to detect and quantitate microbiological targets at very low levels. This work is being accomplished at Utah State University.

#### *7.4.1.1.8 Pathogenic Microbe Sensor Technology*

This research entails developing non-antibody-based capture technology and necessary fluorescence detection instrumentation to detect pathogenic microbes (particularly bacteria) after release in the environment, discriminate between viable cells (including spores) and dead cells, and determine the species of the pathogenic bacteria. Key features of this program include heme-based capture of pathogenic bacteria; pathogen capture using siderophres and carbohydrates; use of peptide libraries to discover new, useful capture ligands; and development of a fluorescence detection device.

Through this initiative, DARPA, working with the Utah State University Research Foundation, plans to develop a cell and protein-capture technology that does not rely on the use of antibodies (which require refrigeration). Fluorescence detection of capture microbes will be achieved without the need for added fluorescent probes, thus allowing it to be used in the scanning of opaque surfaces or products wrapped in clear cellophane packaging, similar to that used in the food industry. It also will allow for the detection of live cells, dead cells, spores, and captured toxic proteins within a matter of one to two minutes through programmed multi-wavelength detection. This will enable users to:

- Rapidly detect the presence of pathogenic bacteria on food surfaces and in drinking water
- More effectively track the movement of plumes of bacteria deliberately released into the atmosphere

- More accurately pinpoint the probable location of a BW laboratory through the in-field analysis of environmental samples.

These technologies have been transferred from Utah State University to B-E Safe, Inc. Negotiations are underway for the manufacture of portions of this technology with two companies well established in the food and medical products markets.

#### *7.4.1.1.9 Novel Antibody Reagents (Immunoplastics) for Sensor Development*

In a collaborative effort between the University of Texas and SRI International, antibodies engineered at the University of Texas are being used to test the analytical capabilities of the upconverting phosphorus flow cytometer developed by SRI International. Their goal is to interface integrated microfluidics technology with antibody-antigen recognition to advance biosensor research, and pave the way for a new generation of robust, ultrasensitive sensors for biological molecules. Requirements that they are hoping to fulfill are those set forth for a military field sensor for BW agents, including sensitivity, accuracy, ease of operation, and the ability to interface multiple sensors to a detection unit. This project is focused on developing a new class of materials, immunoplastics - polymeric materials in which antibodies are directly incorporated within commonly used polymeric matrices. These immunoplastics are expected to drastically increase the utility and stability of antibody diagnostics by providing high antibody loadings, increased stability in storage and in exposure to unconventional environments, and compatibility with the manufacture of solid state devices. Through this research, the team hopes to further advance the production of sensor arrays.

#### *7.4.1.1.10 Tissue Based Biosensors*

This program involves the development and demonstration of innovative cell and multi-cellular tissue-based sensors. DARPA is exploring the use of biological cells and tissues as detector components for sensor devices to report on CB toxins. Through this research, Promega Corporation is trying to develop a capability to detect agents that have not been identified or fingerprinted at the molecular level. The goals are development of biosensors that allow more reliable and accurate assessments of human health risk to operational forces. Key objectives of this program include:

- Enhancing cellular performance for detection
- Improving and extending long-term biocompatibility of materials used in biosensors
- Limiting the degradation of the sensor material under operational conditions
- Improving sampling techniques for the introduction of samples in the field
- Extending operational duty cycles
- Achieving real-time and remote signal processing
- Reducing false positives and negatives
- Extending shelf-life through fluid, frozen or freeze-dried treatments.

#### *7.4.1.1.11 Rapid Sensitive Universal Detection System for Biological Agents of Mass Destruction*

Working in conjunction with DRES, this effort entails the development of a robust system that can identify and isolate specific regulatory elements that are activated in response to the interaction of an eukaryotic cell with biologically-active pathogens. These elements could be used to construct a highly sophisticated biological detection system for biological toxins. The primary goal of this effort is to create a prototype detection system and evaluate its response time, sensitivity, and capability to uncover the biological toxin and its mode of action.

### *7.4.2 Core DoD CB Defense Program*

The DoD CB Defense Program was established to coordinate and integrate research, development, and acquisition of CB defense materiel and systems to support the joint warfighting forces and protect them from the threat or use of CB warfare agents. The program's goals are to provide capabilities that address the highest priority CB threats, from immediate and validated threats through potential far term or emerging threats. It emphasizes a joint Service approach to CB defense RDA in order to eliminate redundancies, leverage common technologies and requirements, and ensure coordination. Capabilities that are to be developed and

acquired are to be based on identified and prioritized requirements and mission needs.

Dr. S. Randolph Long, SBCCOM, briefed DoD biological detection program thrusts at the September, 2000 APBI. He indicated that these thrusts include:

- Point identification – develop fully automated sample preparation and analysis systems for unattended monitoring of air samples and transition it to the JBPDS in FY 2002
- Reagent development – develop improved reagent candidates for implementation in fielded and developmental identifiers via the Critical Reagent Program
- Triggers/detectors – develop technologies which reduce false triggers/alarms by enhancing discrimination against ambient biological background
- Supporting studies – assemble a database of available ambient background data and analyze for key heuristics.

Challenges faced in the point identification area are fluidics, biomarker extraction/cleanup and background interference. To date, the DoD has demonstrated detection of mass and genetic markers of JPBDS requirement levels.

Reagent development concerns include specificity, shelf life and reproducibility. The DoD has demonstrated improved sensitivity of recombinant antibodies verses monocolonals and has initiated an assessment of combinatorial peptides.

In regards to triggers and detectors, the DoD is focused on optical fluorescence and pyrolysis-GC/IMS technologies. They recognize that they need to improve signatures and the current database, as well as devise a system that offers reduced size and weight. However, this requires improvements in current air sampling technologies. The DoD has demonstrated the detection of spores in field tests near requirement levels using Py-GC/IMS.

DoD and DOE are in the process of developing a comprehensive web-based repository of ambient background characterization data gathered from TTCP Chemical, Biological and Radiological Defence Group 10 countries. The DoD Joint Science and Technology Panel for CB Defense has funded SBCCOM to compile and analyze this data and the DOE CB Non-Proliferation Program has funded a special project for the development of the website on which the data will reside. The website is available at http://bioback.ed.ornl.gov. This effort is geared to integrating multiple sources of data and to reducing disparities in collection parameters.

LTC Don Buley, former JPO-BD Deputy Program Manager for Detection, stated that the DoD's detection concept involved layered complementary technologies. He underscored that there currently is "no silver bullet" technology. A non-specific detection capability is needed to distinguish a manmade cloud from a naturally occurring cloud. A generic detection capability also is desired to be able to discriminate BW agents from non-biological particles (e.g., dust). And, a specific detection capability is needed to determine what BW agent combination has been employed.

The DoD hopes to field by 2004 a Joint Biological Tactical Detection System that will provide the capability to detect-to-warn. They envision that this system will entail an affordable, high-density networked array of sensors that is automated with data fusion and linked to identification systems and the warning and reporting network. By 2008, the DoD would like to replace their legacy detection systems with a Joint Modular Chemical Biological Detection System that offers integrated CB detection capabilities and provides point and early warning/detection. Their goals are to significantly reduce the size, weight and power requirements of the system and employ affordable, high-density sensors.

#### *7.4.2.1 Basic Research*

Some of the basic research initiatives being conducted by the DoD in the CB defense area include research into biosensors, aerosol science, and man-portable thin-film detection technology. Their budget in this area is approximately $8.5M.

Biosensor research that has been undertaken involves performing sequencing of high affinity recognition elements and expanding the list of target bioagents. Increases in sensitivity of an immunodetection method using dendrimer bound antibody also was demonstrated. Further efforts in this area are planned, with researchers continuing synthesis of antibody/dendrimer tag

complexes and demonstrating the separation/identification of dendrimer bound antibody/antigen couple via capillary electrophosesis.

Aerosol science initiatives involve assembling and testing a laboratory technology to allow for visualization of changes in growing bacterial cultures as a rapid detection method for bio-active threats. A scattering model theorem and mathematical simplification was completed to allow it to run in reasonable times on small computers for stand-off and point detection of biological particles in the air. Scientists are working to design and fabricate an instrument, based on a scattering model theorem, to measure the back-scatter and image particles. DoD plans to transition this technology to the applied research program for further development.

In regards to thin film detection technology, studies were undertaken on the control of variability in film quality and stability through the use of silane linkages onto piezoelectric materials. Researchers explored the use of shape selective surfaces with attachment of biomolecules and the mechanism for interaction on semiconductor metal oxide sensing elements. Development of this man-portable thin film technology is scheduled to continue, with the focus on optimizing films for both point and cumulative exposure detection applications.

#### *7.4.2.2 Applied Research*

Through the use of applied research funding (totaling approximately $6.2M), several R&D efforts are underway. One project involves implementing a system to automate biological sample preparation procedures for gene-based and mass spectrometric identification/ discrimination of biological materials. This program is scheduled to be completed in FY 2001 with plans to transition the technology to the JBPDS Block II. Methods for release and detection of spore protein markers in 20 minutes by MS have been developed. Several chemical and physical spore disruption methods for Polymerase Chain Reaction detection were evaluated. Plans are to downselect to a single exploratory antibody-based biosensor, FABS. The goal is to automate the sample preparation/processing and to enhance the assay sensitivity to JBPDS requirement levels. A fully automated Biological Sample Preparation System coupled with a gene probe sensor and next-generation mass spectrometer is to be demonstrated.

In regards to biological point detection, force differentiation analyzer assays for simulants at or near sensitivity goals for fielded biological identifiers were developed. A new integrated waveguide approach and molded fluidics assembly for multiagent immunosensors were implemented. Several commercial and developmental biosensors were tested at JFTs.

Several biological early warning detection projects are underway. Researchers have downselected among potential fluorescence based triggers/detectors. Candidate new stand-off biodetection approaches are being evaluated. A linear response to particle size has been demonstrated using single particle fluorescence studies.

A couple of different approaches for arrayed detector networks are being pursued. Efforts to enhance reliability (false detection reduction) and increase discrimination capability of optical analyzers by adding shape/size analysis have been initiated. PY-GC-IMS is being examined as a potential JMCBDS candidate, with the emphasis on ascertaining this technology's ability to determine the chemical identity of signature markers for simulant bioagents.

A prototype human super-library of antibodies representing the entire human immune response is being established. Work continues on development, test and transition of new recombinantly-derived antibody-based recognition elements. In addition, a botulinum toxin recombinant antibody was tested, using dendrimer support on a ticket format, that demonstrated improved performance over standard ticket implementations. Future work in this area entails assessing recombinant antibodies using biosensor testbeds, evaluating methodologies for turn-around time to develop new antigen binding fragments from unknowns, and evaluating combinatorial peptides as alternative recognition molecules.

Work has progressed on next generation biological detection systems as well. Three BIDS inlets and two JBPDS Block I inlets were characterized. Technical approaches to advanced aerosol collector and inlet technology were identified to overcome technical shortcomings of existing equipment. A

transportable aerosol containment sleeve with supporting controlled aerosol generation was deployed to field tests for stand-off biodetection development.

In work to design a man transportable detector with low power and no field maintenance requirements, polymer film chemistries and advanced Semiconducting Metal-Oxide (SMO) arrays for detection of CB agents were explored. Polymer coated surface acoustic wave and chemiresistive conducting devices that are sensitive and selective to nerve, blister, and blood agent simulants are being developed. Impedance and fluorescence-based biosensors employing immunological and DNA detection probes are being researched. And, integration of hybrid sensor array devices and electronics, neural networks, and other data acquisition and display hardware/software into a prototype detection system has been undertaken.

Some novel bio sensor concepts are also being explored. These include currently fielded non-CB sensors that can provide CB use signatures, such as radar and acoustic sensors, as well as chemical stand-off approaches to bio detection through optical signatures not presently employed. Point sensors having simultaneous CB detection capabilities will be identified. Networking of disparate sensors is to be accomplished through emerging information management processes.

Developing technologies for highly multiplexed identification of biological agents implemented on platforms such as PCR and flow cytometry are planned. Researchers hope that this effort will greatly expand the number of agents identifiable in ensemble identification suites that will be transitioned to JBPDS and to upgrades of fielded systems. This effort will also develop the capability to characterize unknown biological agents.

#### *7.4.2.3 Advanced Technology Development (ATD)*

The biological detection projects fall under the ATD budget lines and are geared to demonstrating CB defense technologies in an operational environment. Funding for CB defense under the ATD budget lines is approximately $57M and for counter-proliferation support is $10.5M in FY 2000.

These programs entail conducting proof-of-principle field demonstrations and tests of system-specific technologies to meet specified military needs. Work conducted under this budget line transitions to and provides risk reduction for the Demonstration/Validation and Engineering and Manufacturing Development activities. The ATD receives funding through this PE. The goal of this ATD is to fabricate, demonstrate and integrate advanced point and stand-off detection technologies. Other programs funded under this budget line include the Small Unit Biological Detector (SUBD), the Joint Service Warning and Identification LIDAR Detector (JSWILD), the Joint Chemical/ Biological Agent Water Monitor (JCBAWM), and the CB Individual Sampler.

Phases I and II of the SUBD were scheduled to be completed in FY 2000. The culmination of these phases is delivery of an engineering prototype consisting of: a collector/concentrator that samples the air and concentrates biological aerosols into a fluid media suitable for analysis, and a biosensor that analyzes the collected samples and identifies the biological agent in five to ten minutes by using immunoassay technology. Researchers are also to assess the military utility of using small-scale sensors to detect biological or chemical agents in near real-time and relay that information to tactical elements.

Another ATD initiative, the Counterproliferation Program, is designed to accelerate delivery of new tools, equipment and procedures to combat forces. Programs funded under this ATD budget line includes the Biological Detection (BIODET) initiatives, Biological Non-Systems (BIO Non Sys) efforts, and the Critical Reagents Program (CRP).

In support of the BIODET program, advanced material technologies developed for the miniaturized environmental air sampler and concentrator for biological materials were transitioned to the combined aerosol sampler and detector. Scientists also continued development of advanced technologies for high sensitivity CB agent detection using broadband, miniaturized mass spectrometer techniques and upconverting phosphor technology development for miniaturized flow cytometer biological agent detection prototype. Upcoming plans in this area are: to develop a biological identification system using nucleic acids to allow for a less expensive

and broader biological detection capability, to transition upconverting phosphor technology development for a miniaturized flow cytometer biological agent detection prototype, and to complete the first generation of a Biological Time-of-Flight Mass Spectrometer for transition to field testing.

Biological non-systems work involved collecting background aerosol particle data and liquid samples for identification of potential battlefield interferents at Outside the Continental United States (OCONUS) fixed sites. Future work is aimed at development, testing and evaluation of automated sample preparation technology for PCR devices and development of non-specific detection, multiplexed assays and associated reagents.

CRP work entails developing reagents (antibodies and antigens) that are critical to the development, testing, and support of biological detection systems.

#### *7.4.2.4 Demonstration and Validation*

The budget line for demonstration and validation supports PDRR of CB defense equipment, including chemical/biological/toxin detection and warning systems and transition of biological detection components – early warning, collector concentrators, generic detection, and improved reagents – for the future JBPDS Block II. PDRR can facilitate transition of technologies by testing them for suitability in the relevant development program. It also supports the CRP for the development of advanced reagents for legacy and future detection systems. Biological defense funding under this budget line was approximately $68.5M for FY 2000; for contamination avoidance, $4M; and for counter-proliferation support, $14.6M.

A primary focus of this budget line is on the JBPDS Block II system, finalizing the design and assessing candidate components. Another key initiative is legacy system upgrades. These efforts are aimed to improve detection time and reduce operation consumables. Some of the technologies under consideration are the TOF MS/MS and Ultraviolet Triggers.

Under the Counterproliferation Support budget line, the focus is on fulfilling the requirement of providing full dimensional protection to deployed forces and critical fixed sites, such as Aerial Ports of Debarkation and Sea Ports of Debarkation. The project supports the accelerated fielding of operational capabilities to CINCs through the ACTD process. It also funds the development of the LR-BSDS.
The JBREWS ACTD's aim is to provide early warning, detection, and identification of BW agents at fixed sites. The system will be comprised of distributed BW agents' sensors with a remote capability that will be compatible with legacy BW detection systems.

Funding for the LR-BSDS has gone to completing fabrication of the first two systems, conducting testing, and continuing fabrication of Initial Operational Test and Evaluation systems. Further details of the above mentioned systems are provided in Section 5.

#### *7.4.2.5 Engineering and Manufacturing Development*

This budget line supports the Engineering and Manufacturing Development of CB defensive equipment, both medical and non-medical. These projects have been restructured to consolidate Joint and Service-unique tasks within four commodity areas: contamination avoidance, force protection (individual and collective), decontamination and medical countermeasures. The estimated budget for FY 2000 was approximately $118.5M, with some $59M allotted for contamination avoidance, $15M for biological defense, and $5.6M for counter-proliferation support. Other programs funded under this budget line include collective protection, decontamination systems, individual protection, medical biological defense, and medical chemical defense.

Biological detection efforts under this program are geared towards providing theater protection through the development of point and stand-off detection systems. Initiatives funded under the biological defense budget item include JBPDS, an integration of the U.S. Army's BIDS, the U.S. Navy's IBAD and USAF and USMC Service-specific development programs. It is supposed to be capable of identifying, within 15 minutes, BW agents listed in Category A of International Task Force 6 Report. The system is to be integrated into each Service's platform (e.g., HMMWV, ship, truck, etc.) or airbase or port to provide a common detection capability with joint interoperability and supportability. The JBPDS is designed to increase the number of agents that

can be identified by the BIDS and IBAD systems; provide automated, knowledge-based, near real-time identification; and provide a first time point detection capability to the USAF and USMC. The program is structured into two Block Engineering and Manufacturing Development phases: Block I will provide the Services with an automated BW agent identification capability, and Block II will upgrade the Block I production suite to more fully comply with the Joint Operation Requirements Document by taking advantage of technology advances from the technical industrial base. Block II efforts include reducing system size and weight, as well as development and integration of advanced dry detection/identification technologies to reduce life cycle costs and logistics demands. Block II will advance biological point detection from the operational level to the tactical level (i.e., smaller, low-powered devices employable by front-line units).

Funding also is allotted to the CRP, to integrate and consolidate all DoD reagents, antibodies, and DNA biological detection requirements in demonstration/validation through production. The CRP is supposed to ensure the availability of high-quality reagents throughout the life-cycle of all BW detection/identification systems. It supports all aspects of manufacturing scale-up of development protocols for CRP-developed products.

A number of programs have received funding through the Contamination Avoidance budget item. These include the CB Mass Spectrometer (CBMS) II, the JSLNBCRS, the JWARN, and the Nuclear, Biological and Chemical Reconnaissance System (NBCRS) Block II.

The CBMS II is to replace the MM1 Mass Spectrometer. Developers envision that the CBMS II will offer significant enhancements by simultaneously detecting and identifying CB threat agents at lower system cost. The JSLNBCRS is a new lightweight NBC detection and identification system and will consist of a base vehicle equipped with hand-held, portable and mounted, current and advanced NBC detection and identification equipment. It will provide on-the-move reconnaissance and surveillance in support of combat, combat support, and combat service support forces. There will be two variants of the JSLNBCRS – the HMMWV and the Light Armored Vehicle (LAV). The NBCRS is a nuclear and chemical detection and warning system, with biological sampling equipment integrated into a high speed, high mobility armored carrier capable of performing NBC reconnaissance on primary, secondary, or cross country routes throughout the battlefield. Block II improvements to this system will ensure that the system will meet all of the requirements contained in the approved requirements document.

The JWARN will provide integration and analysis of NBC detection information with Command, Control, Communications and Computers Information and Intelligence on the battlefield, automating the NBC warning and reporting processes currently performed manually throughout the Services. JWARN is being developed for deployment with NBC detectors in the following battlefield applications: combat and armored vehicles; tactical vehicles; vans; shelters; shipboard applications; area warning; semi-fixed sites; and fixed sites.

Further discussion and clarification of the systems mentioned above were provided in Section 5.

### *7.4.3 Joint Initiatives Conducted by Service R&D Establishments*

Service R&D establishments are conducting specific joint biological detection initiatives. Some of these efforts are highlighted in the following paragraphs.

#### *7.4.3.1 U.S. Army Led R&D Programs*

##### *7.4.3.1.1 Miniaturized Sample Preparation Module*

One effort that the U.S. Army is researching is the development of sample preparation modules to interface with Micro-Electromechanical Systems (MEMS) and micro-electronics sensors. MEMS technology has reduced the size and power requirements for detection of genetic material from microorganisms using PCR. To date, however, portable detectors are suitcase size and require manual addition of a single colony culture. A remaining challenge is the miniaturization of sampling devices. Such devices could be for aerosols, water sampling or soil samples that could be interfaced with existing MEMS sensors. Much development is

required in the area of automated sample cleanup since the purity of DNA in small devices is much more critical.

In Phase I, the U.S. Army will catalogue current sample collecting or sample preparation systems and evaluate them for miniaturization. One or more devices will be selected along with MEMS sensor(s) into which it will interface.

Phase II will entail constructing and demonstrating a miniaturized device. Integration with the MEMS sensor device(s) selected in Phase I will be conducted for development of a complete biosensor system ready for field tests.

In Phase III, the U.S. Army intends to pursue dual use applications, envisioning that development of miniaturized sample collection and preparation devices would greatly accelerate commercialization. Such devices could be used in medical, environmental monitoring and food preparation areas.

#### *7.4.3.1.2 Improved Sensitivity for CB Stand-off Detection*

The U.S. Army is pursuing new approaches for increasing the sensitivity of LIDAR stand-off CB detectors. Current LIDARs for stand-off CB detection uniquely identify CB agent spectral features by measuring the laser energy at each wavelength that has passed through the cloud. The ultimate sensitivity of the measurement depends on the signal to noise ratio. Current LIDARs are capable of detecting small fractions (1/10 to 1/100) of the dose that will produce a lethal effect in 50% of poisoning cases. However, even this sensitivity is not sufficient to determine completely safe boundaries for areas that have been under attack (due to long-term effects of low-level exposures). Nor is it sufficient to measure suspected areas of weapon manufacture. In order to do these tasks, the sensitivity must be raised by about a factor of ten. Current carbon dioxide ($CO_2$) LIDARs using Transversely Excited Atmospheric (TEA) lasers can attain 2% noise levels consistently by pulse averaging. The desired factor of a ten sensitivity increase can only be accomplished by reducing the noise of the system by a factor of ten. Among the methods being considered are:

- signal averaging to levels consistently less than 1% noise with a goal of 0.1%
- further reducing instrument noise, such as with lower noise detector elements and/or preamplifiers, better energy normalization techniques, and better shielding; and
- coherent detection, including the possibility of utilizing detector arrays in order to reduce transmitter repetition rate requirements.

If the approach is successful, the U.S. Army envisions it could be integrated into the $CO_2$ TEA laser-based JSWILD acquisition program to enhance its CB detection capabilities to include very low level detection.

Military applications include full-sized and miniature stand-off CB detectors for contamination avoidance and decontamination. In addition, dual-use intelligence and domestic preparedness applications could directly benefit from having a stand-off detection device with greatly increased sensitivity. Commercial applications include spin-off detectors for stand-off environmental pollution monitoring and for drug interdiction.

#### *7.4.3.1.3 Detection and Identification of Buried or Concealed BW Agents and Simulants Using Nuclear Quadrupole Resonance Spectroscopy*

The U.S. Army is pursuing developing a sensor for detecting BW agents and simulants using Nuclear Quadrupole Resonance (NQR) Spectroscopy. Such a system would be capable of detecting concealed BW agents in a closed suitcase or buried below the ground.

NQR has been used for detection of explosives, narcotics, and buried landmines. Commercial NQR detection systems are now beginning to appear in airport security systems for detection of explosives, narcotics, and other contraband. Typically in these applications, the NQR signal of a nitrogen-14 atom in an unusual molecular configuration is detected. All compounds that contain nitrogen-14 have NQR absorption bands in the region of 0.2 MHz to 5.0 MHz. Electromagnetic radiation in this region can easily see through a suitcase to look for contraband or look below the surface of the ground for buried landmines. Dipicolinic acid is a major constituent of the bacterial spores that make up many BW agents. In some cases, calcium dipicolinate constitutes up to 17% of the dry weight of the spores. Dipicolinic acid is believed to be an important contributor to the

resistance of spores to both heat and UV radiation. The material also appears to be important in spore stability and spore germination. The dipicolinate ion has a nitrogen atom in a benzene ring and has a distinctive NQR signal. The NQR signatures of dipicolinate have been predicted to lie somewhere between 3.0 and 5.0 MHz.

In Phase I, the U.S. Army intends to demonstrate on a laboratory scale a proof-of-concept NQR system capable of detecting bacterial spores. The proof-of-concept system shall be able to detect small (less than ten grams) quantities of the BW simulant Bacillus Subtilis using the distinctive NQR signature of the bacterial spores.

In Phase II, researchers will build a prototype of an NQR system for detecting BW agents in a closed container, such as a suitcase. They will demonstrate detection of small quantities (less than ten grams) of Bacillus Subtilis in a closed and locked suitcase.

Phase III will entail conducting a feasibility study of modifying existing contraband detection systems in airports based on NQR spectroscopy to provide additional protection against BW agents shipped in closed containers.

#### *7.4.3.1.4 CB Water Monitor Biological Concentration*

The U.S. Army is also focused on building a hand held system to collect, concentrate, and isolate biological agents from source, treated, stored, and distributed water supplies. The extracted agents will be presented to varying detection systems. Analytes include bacterial cells, spores, cysts, viruses, and toxins. Novel methods to extract genetic material, including DNA and mRNA, also are desired.

The Joint Service Agent Water Monitor (JSAWM) will require sample collection, concentration, and extraction of biological analytes such as bacterial cells, spores, cysts, viruses, and toxins. No detection technology has been found that can detect to the trace levels required and accommodate the widely varying background waters (source, treated, stored/distributed) that will be monitored. Target analytes may be diluted in large volumes of water (thousands of gallons), the water may be turbid (such as natural waters), and it is likely the background of the water will not be favorable to a particular sensor technology. This is especially true for natural water samples.
This pre-processing is seen as a separate technology "module" in the JSAWM system. After a sample has been collected, concentrated, and extracted, it can be passed to a number of competing and/or complementary detection technologies.

The capability to extract, concentrate, and isolate trace levels of biological agents from thousands of gallons of water currently does not exist in the commercial market. The proposed pre-processing system would have immediate applications in monitoring municipal and commercial water supplies for possible contamination by biological contaminants.

#### *7.4.3.1.5 CB Water Monitor*

As mentioned above, the U.S. Army is looking to develop a hand held, real time sensor system that can detect, identify, and quantify CB agents in water supplies. Both in-line and batch monitoring are desired. Time to detection sought is ten minutes. Water supplies include point source, treated, stored and distributed waters. The goal of this sensor system is to provide early warning of contamination or possible attack.

There is an immediate need for the ability to detect, identify, and quantify CB agents in water supplies during water point selection, production, storage, and distribution to consumers (including shower points and personnel decontamination stations). Water point selection can be natural waters such as lakes, rivers, streams, reservoirs, municipal waters. Production water is field/shipboard treated water. Stored and distributed water can be treated field water, bottled water, and locally purchased water that is trucked or piped to military storage (field and ship).

Biological detection by class is considered to be bacteria, virus, toxin, parasite and pathogenic versus non-pathogenic. The trace levels of detection required are considered problematic. The U.S. Army is examining technologies to concentrate and extract dilute analytes from water. However, they would like to have the ability to detect, identify, and/or quantify without such pre-processing.

The current estimated requirement is 20,000 fielded units for Joint Service use. Candidates

that the U.S. Army selects will be added to the JSAWM as a "technology module". The JSAWM concept is a modular system analogous to current day computers. Peripheral devices can be added and removed by the user as needed. Additional applications exist in other DoD and government agencies such as Medical, Domestic Preparedness, Demilitarization, and Treaty Verification. JSAWM has been working closely with Center for Ecological Health Research (CEHR) where a medical requirement for water monitoring exists. Although JSAWM and CEHR requirements have striking differences, they are working together to leverage technologies and programs where possible. In addition, the proposed water monitor would have immediate application in monitoring municipal, commercial, and recreational water supplies.

The Environmental Protection Agency (EPA) has funded two non-profit organizations in the past two years to search for advanced warning and early monitoring technologies for water contamination. At a recent workshop of public water managers, the Center for Disease Control (CDC), and the EPA, participants addressed the concern and need for early warning monitoring of public water. The EPA has an immediate need for early warning monitoring of recreational water.

#### *7.4.3.1.6 Development of a Miniaturized Biological Detector*

The U.S. Army is seeking to develop a miniaturized biological detector that can be either scattered on the battlefield for early warning or worn by the soldier on his/her lapel. The ECBC is developing a number of biological agent detectors. All the detectors that are currently under development are large, heavy, and require expertise in their operation. Some detectors that are in development are briefcase size, but require a separate sampler for collecting the particles from the air and introducing them to the detector.

In recent years, great progress had been made in the micro machining/nano technology area. The purpose of this effort is to apply this emerging technology for developing a miniaturized biological detector that will include a miniaturized aerosol sampler/collector and detection device, a miniaturized GPS and a miniaturized communication system. The detector can be either a general biological detector or detectors for specific agents. In the first case, multiple miniaturized detectors could be scattered on the battlefield and act as an early warning system by networking them. In the second case, the detector would be worn by the individual soldier to alert him/her and the medics when he or she was exposed to the specific agents.

The U.S. Army believes that this device will greatly facilitate treatment. In addition, the detector will be able to detect when the soldier is being exposed to a locally endemic biological material. The detector should be able to operate as a stand alone device for a period of at least 48 hours unattended operation, stay in constant communication with the home base and store the data (including GPS data and time/date) for that period.

In its military application, the detector can be used as an early warning system when scattered on the battlefield ahead of the troops (Army and Marine Corps) around an airfield (Air Force) or a port (Navy). Another potential military use is to fly the miniaturized detector into a suspicious cloud, or drop it by a parachute into the cloud to identify the nature of the cloud and determine if it can present a hazard to the troops.

This technology could be transferred to the health industry, the environmental protection arena and the industrial hygiene area. A miniaturized biological agent detector can be used by health care providers to monitor the spread of infectious diseases and by individual health providers to detect when they were exposed to an infectious organism. In the environmental protection arena, it will enable the EPA to quickly identify the cause of a "sick building". Industrial hygienists would be able to use the device to monitor and control the exposure of workers to harmful organisms.

### *7.4.3.2 U.S. Navy Led R&D Programs*

#### *7.4.3.2.1 CB Sensor for Munitions*

The U.S. Navy is trying to develop a CB sensor system that is robust and small enough to be used as payload in an artillery projectile. There is no remote CB sensor that can be deployed from a ship prior to expeditionary forces being projected ashore. The intent of this project is to develop the technology that will lead to a quickly deployed CB agent early warning capability to

support forces participating in amphibious operations. Specifically, the researchers hope to develop the agent detection subsystems of a CB detector payload for a 5"/62 gun projectile, which can be delivered by Naval guns for quick and accurate placement on the beach. They would like to design a system that is rugged enough to withstand the forces associated with delivery (14,000 gs/300rev/sec), miniaturized to fit within size (290 cubic inches) and weight (18 pounds) constraints and able to communicate alerts back to ships 23 miles off-shore. The subsystem desired would be capable of performing simultaneous analyses of multiple CB agents.

These sensors would have significant potential for airport inspection applications and for remote sensing in public areas such as subway stations. Additionally, the U.S. Navy believes that miniature automated titration analysis systems that can be manufactured in large numbers would be of significant interest to educational institutions and commercial chemical and pharmaceutical companies.

#### *7.4.3.2.2 Development of a Portable Aerosol Collector*

The U.S. Navy is exploring development of a badge-sized aerosol collector using either electrostatic or electrodynamic precipitation to monitor personal exposure to BW agents. Researchers are seeking to apply the same technology to develop a small sampler that could be used to monitor areas within ships, aircraft cockpits and cabins, and access repair panels for evidence of contamination.

Personal detection for troops operating in the field at remote locations close to front lines or in high threat areas is lacking. In addition, viable technologies to monitor for contamination within or on aircraft, within ships and other assets also are lacking. For personal/small area detection, the U.S. Navy researchers want an aerosol sampler that is lightweight, reliable, quiet, capable of operation without the use of battery packs or bulky pumps and little or no fluids. They are looking to develop a portable sampler based on electrodynamic precipitation technology coupled with microelectronic manufacturing techniques. Electrodynamic precipitation works by establishing an electrical gradient that attracts particles.

The researchers believe that electrodynamic sampling technologies have numerous advantages over more conventional sampling devices, such as prolonged sampling, have no moving parts, and require no fluids to capture aerosol particles. When combined with microelectronic manufacturing, small samplers requiring very low power requirements can be manufactured. Such a sampler could be used for monitoring an individual's exposure or that of a small area.

Dual use of such a sampler includes measuring exposure of those working in Biosafety Containment Level 3 and higher laboratories for exposure to dangerous microorganisms. It could also measure exposure of workers to dust, soot and other particulates encountered in potentially hazardous working environments such as mines, metal working shops and slaughterhouses. The sampler would also be useful in assessing the hazards found in compartments that need "gas-free" testing prior to entrance and for collecting trace amounts of illicit substances.

#### *7.4.3.2.3 Particle Filter/Separator For Use In Biological Samplers*

The U.S. Navy’s goal in this program is to develop reliable in-line filters and separators for use with biological sampling equipment at 800-1000 liters per minute flow rates. They hope to prove the feasibility and reliability of incorporating such devices into existing systems without reducing down stream function.

There is a need to reduce the ingestion of 50 micron and larger particles from a biological wet sample. This need has arisen due to large particles causing clogging of particle counters (e.g., TSI APS and MetOne) and wet samplers in current biological agent detection systems. The base line particle sizes for biological agents are 2-10 micron with some clusters as large as 15 micron and as small as one micron. Background particles are found at many sites employing developed biological agent detection systems. These large particles have many sources and when the devices are constantly exposed to these sources, clogging occurs. Some examples of sources are industrial waste and equipment exhaust as well as pollutants from controlled and uncontrolled burns (e.g. forest fires, controlled back-burns and the burning of old crop fields).

The filter/separator would be placed on existing biological sampling equipment used on U.S. Navy ships. This would include the IBAD. It could be used with the JPO Portal Shield and JBREWS equipment. These devices also would have a use on future aerosol sampling equipment, where applicable. They could also be used within the civilian community in conjunction with biological detection equipment for anti-terrorism.

#### *7.4.3.2.4 Field Rugged Man-Portable CB GC MS for Environmental Assessments*

The U.S. Navy is looking to develop a capability to assess federal, state, and local landfills, CB storage facilities (bunkers, ammunition dumps, etc.), and petroleum and chemical plant tanks (above and below ground), for surface contamination.

The intent of this project is to rapidly assess CB agents that permeate (filter) from stored 55-gallon drums, tanks, landfills, or pipelines in petroleum plants. The researchers desire a CB GC/MS that is capable of performing simultaneous analysis of multiple CB agents, as well as Toxic Industrial Chemicals (TICs) and Toxic Industrial Biologicals (TIBs).

The Field Rugged CB GC/MS could be used by federal, state, and local authorities to monitor landfills, wetlands, petroleum and chemical plant discharge (holding pits, ditches, etc.), and CB storage bunkers for CB threat agents seeping into ground water, or rising to the surface. Developers envision that the overall system cost would be reduced if it were manufactured in large numbers. This would give both DoD and civilian authorities the capability to accurately assess TICs, TIBs, and CB agents of interest.

#### *7.4.3.2.5 Force Differentiation*

NRL is developing a sensor capable of detecting biological species such as cells, proteins, toxins, and DNA at concentrations as low as $10^{-18}$ moles. The Force Amplified Biological Sensor (FABS) takes advantage of the high sensitivity of force microscope cantilevers to detect the presence of as little as one superparamagnetic particle bound to a cantilever by a sandwich immunoassay technique. The device performs an assay in about ten minutes. Lock-in detection and the use of a reference cantilever provide a high degree of vibration immunity. Using this technology, an array of ten or more cantilevers can be implemented, which provides greater sensitivity and the capability to detect multiple species simultaneously. The force amplified biological sensor also offers the potential of distinguishing and studying chemical species via its ability to measure binding forces.

FABS uses a sandwich assay, in which antibodies against a particular protein, virus, or bacterium are covalently bound to a solid surface. The sample solution flows over the surface, and the antibodies capture any of the virus present. Next, superparamagnetic beads, also coated with an antibody against the virus, flow through the liquid cell and bind to the analyte. After washing away excess beads, a number of beads remain bound to the surface through the virus. After the beads become bound to the cantilever, an electromagnet is turned on. The magnetic field pulls on the beads, which pull on the cantilever and make it bend. The cantilever-beam force transducer senses the presence of the magnetic beads, the number of which is proportional to the concentration of analyte in the sample. By determining the number of beads, researchers can calculate the concentration of virus in the original sample.

Atomic Force Microscopy (AFM) technology provides a number of ways to measure the bending of the cantilever. Currently, NRL is using piezoresistive cantilevers. Unlike the optical detection methods commonly used in AFM, piezoresistive cantilevers do not require external sensing hardware. This is an advantage for FABS, since such hardware usually requires manual alignment to the cantilever and tends to be large and easily damaged.

Unlike AFM, FABS does not have a scanning element, feedback, or tip–sample approach. The only element that FABS has in common with AFM is the cantilever.

### *7.4.3.3 U.S. Marine Corps Led R&D Program*

#### *7.4.3.3.1 Small Unit Biological Detector (SUBD)*

The SUBD, a USMC Service-unique requirement, will be a low power, manportable biological detector for use by the USMC CB Incident Response Force. The SUBD designers are developing second generation aerosol

collection and identification technologies that will be integrated into a smaller system with more reusable components that could offer technology enhancements for the JBPDS program. The requirements for this system are that it weigh less than 80 pounds, be less than 2.3 cubic feet, need less than 150 Watts of power, and be able to identify 12 BW agents within 20 minutes.

Benefits that researchers are hoping will be gained by this system are improved detection and identification capabilities and the ability to perform real-time analysis of agents, communication of exposure information to command centers, and increased battlefield awareness and intelligence. Identifier and collector component development and system integration was scheduled for FY 2000. Prototype development is slated for the FY 2001 timeframe. The program schedule hinges on adequate funding.

#### *7.4.3.4 U.S. Air Force Led R&D Program*

##### *7.4.3.4.1 Discrimination of Biological Agents at Stand-off Distances*

The USAF is looking to develop and demonstrate a novel eyesafe, manportable, laser-based technique to discriminate biological agents from naturally occurring backgrounds at moderate stand-off distances (up to 10 km). Possible detection techniques under consideration include, but are not limited to, discrimination of the bio particles by utilizing polarization and/or multiple scattering methods.

The current state-of-the-art biological detection system is the M94, a helicopter-mounted one micron scattering detection lidar. The eyesafe upgrade to this device is the LR-BSDS. Both detect the presence of aerosol clouds at ranges of as great as 30-50 km. However, neither device is capable of discriminating between naturally occurring aerosols and those associated with a BW release.

Using another LIDAR technology, the SR-BSDS is currently being developed for evaluation. This device will be able to detect the presence of biologically-active particles within a naturally occurring aerosol environment. However, it utilizes non-eyesafe ultraviolet light, is severely limited in range, is quite large, and must be operated in darkness for maximum sensitivity. For example, it has been calculated that detection ranges at night are less than 1 km for minimum threat clouds. In addition, both the SR-BSDS and LR-BSDS are very large and expensive systems, weighing over 1000 pounds each, and require a dedicated vehicle such as a helicopter or HMMWV to house them and provide the power they need.

Naturally occurring atmospheric particles fall into the 0.3 to 0.7 micron range. On the other hand, particles onto which BW agent have been deposited are much larger (two-ten microns). If radiation impinges on clouds of these materials, the larger particles will cause the radiation to be multiply reflected. Thus, it is possible to discriminate clouds of BW agents by measuring the relative amounts of the singly and doubly back-scattered signals. USAF researchers believe this can easily be done by using a LIDAR with detectors that are viewing both on and off axis.

In addition, researchers know that the particles onto which the biological agents are deposited are cylindrical (or at least non-spherical) in shape, thus raising the possibility that they will be sensitive to polarized light. Since it has been established that naturally-occurring dust has no such polarizing qualities, USAF researchers believe there is a possibility that this fact can be used to discriminate biological particles from the atmospheric background. Preliminary calculations show that identification of the biological aerosols could be possible at ranges up to 10 km if either of these techniques prove viable.

Military applications include manportable, stand-off CB detectors for contamination avoidance, decontamination, and counterproliferation. Commercial applications include detectors for stand-off environmental pollution monitoring.

### *7.4.4 Technical Support Working Group (TSWG)*

The TSWG is an interagency team funded mostly by DoD. It is made up of representatives from eight federal departments - Defense, State, Justice, Transportation, Treasury, Federal Emergency Management Agency, Public Health Service, and the Central Intelligence Agency - and over 50 agencies. The Group conducts counter-terrorism technology R&D and prototyping, focusing on explosives detection and technologies that will detect and protect

against WMD terrorism. The TSWG coordinates and manages the National Counter-terrorism Research and Development Program, known as the Counter-terror Technical Support (CTTS) Program. The CTTS is a fast track R&D program that addresses domestic and international aspects of terrorism. CTTS projects are selected to meet the requirements identified and coordinated, through the TSWG, with other U.S. agencies and three countries: Israel, Canada, and the United Kingdom.

#### *7.4.4.1 TSWG Biological Detection Technology Development Initiatives*

The CTTS program is executing projects at both the national and international level to support first responders at Federal, state, and local levels. Their R&D efforts include the following:

- Improvised Agent Evaluation - Determine the effectiveness of existing detectors and countermeasures against improvised agents and improvised dissemination methods.
- CB Containment Vessel - A bomb containment vessel that is designed to contain CB agents, as well as explosives and fragmentation. This is similar to the total containment vessel that the U.S. Capitol Police use. The Capitol Police is the proponent for this effort.
- Sampling Development Capability - Aids the first responder in gathering samples of air, water, and soil for later analysis for the presence of CB agents. The TSWG and National Institute of Justice are jointly developing this capability.
- Detector Evaluation - Evaluation of existing chemical agent detectors against the threat agents most likely to be encountered by first responders. This is a joint TSWG-NIJ effort that relies on a threat study that TSWG is conducting in cooperation with the National Institute of Justice.
- Non-Intrusive Detection of Chemical, Biological and Explosive Threats - Determines the contents of closed containers by using digital x-ray processing, thus not disturbing the contents of the containers. The real-time radiography, or the RTR–4, is a system used by bomb squads or other emergency responders to determine the contents of a suspicious package. This system will provide responders with information to help identify whether a threat is an explosive device, a chemical agent, or a biological agent.
- CB Overpack Bags - A low cost containment device for suspect chemical or biological devices. This system will be commercially available in the next nine months. When suspicious material or a device is discovered and is determined it can be moved safely, the item would be placed in the bag for proper transport and then for further analysis. It was originally asked for by Special Operations forces. It is now available to the USMC Chemical Biological Incident Response Force, state and local first responders as well as the U.S. Army tactical escort unit.

In addition, the TSWG is conducting joint testing with international partners to develop CB mitigation equipment and techniques that maximize use of existing and planned equipment in state and local response unit inventories. Also, the TSWG is working with the Federal Transit Administration to determine the effectiveness of detection and response procedures in urban settings, such as a subway.

### *7.4.5 Department of Energy*

#### *7.4.5.1 CB Nonproliferation Program (CBNP)*

DOE's CBNP was initiated in FY 1997 in response to the Defense Against Weapons of Mass Destruction Act ("Nunn-Lugar-Domenici"). The mission of the CBNP is to develop, demonstrate, and deliver systems and the supporting technologies that will lead to major improvements in the U.S. capability to prepare for and respond to chemical or biological attacks. Their FY 2000 budget is $40M; a $21.5M increase over their FY 1999 budget. Their request for FY 2001 is $42M.

The DOE Office of Nonproliferation Research and Engineering (NN-20) conducts applied research, development, testing, and evaluation—and leverages the work of others—to produce technologies that lead to prototype demonstrations and detection systems. Development is focused on technologies for which the basic science is already understood. The program targets major capability enhancements that can be achieved in the three- to five-year timeframe, not incremental

improvements. The DOE program currently has four areas of specific focus: detection, biological foundations, modeling and prediction, and decontamination. Technologies that are developed are delivered to government users or commercialized.

Their activities are divided into four program areas: proliferation detection, proliferation deterrence, nuclear explosion monitoring, and CB nonproliferation.

Objectives include:

- Remote detection of the early stages of a proliferant's nuclear weapons program
- Location, identification, and characterization of nuclear explosions underground, underwater, in the atmosphere, and in space, to enhance the U.S. nuclear explosion monitoring capability
- Satellite-based nuclear explosion sensor systems
- Technologies for nuclear materials protection, control, and accounting; monitoring nuclear warhead dismantlement; counter-nuclear smuggling; and law enforcement forensics, and
- Detecting the proliferation or use of CB agents, and minimizing the consequences.

#### *7.4.5.2 Domestic Demonstration and Application Programs*

DOE undertakes Domestic Demonstration and Application Programs (DDAPs) to integrate individual technologies into systems in a two- to three-year timeframe. DDAPs are focused on demonstrating the potential impact of a technology, integrated into a system, to address specific problems facing a CB Urban Defense System. The goal of their DDAPs is to integrate current technology into prototype operational systems directed at specific applications. DOE also uses the DDAPs to introduce emerging technologies and limited capability systems into operational settings, giving system operators experience with the technology. There are two DDAPs currently underway: PROTECT: Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism and BASIS: Biological Aerosol Sentry and Information System. Both of these programs focus on the demonstration of early detection, identification, and warning systems. These DDAPs also require the development of interfaces between the detection systems and the command and control systems that will be tasked to take action based on detection system data. The duration of these DDAPs is expected to be two to three years (DOE's typical DDAP timeframe objective), with follow-on demonstrations as required. The PROTECT DDAP examines vulnerable facilities that have high concentrations of people, with subway systems as an initial focus and airports as a secondary emphasis.

The BASIS DDAP is geared for monitoring of airborne biological agents during a special event, such as sporting events, political conventions and international summits, or for a period of heightened alert. A key goal of the BASIS initiative is to develop a biological early warning system to provide first responders with reliable detection and identification data of a biological aerosol attack. Several Distributed Sampling Units are being used to provide wide area coverage to monitor aerosols and collect samples for analysis. These samples are then provided to a mobile field laboratory for high-throughput analysis. A system prototype integration test is scheduled for FY 2001, followed by a DDAP demonstration.

#### *7.4.5.3 Domestic Counter Terrorism Efforts*

The DOE national laboratories are working together to develop a modular system of sensors for CB sensors to counter domestic terrorism. Their goal is to develop a suite of portable instruments that, when operated together, will enhance the area coverage and lower the false alarm rates.

DOE noted that the needs of domestic counterterrorism differ in some respects from battlefield protection objectives. Among the differences they pointed out are that domestic counterterrorism deals with a much broader range of agents and has more demanding false positive requirements. In addition, detecting an attack in an urban population will be more difficult as there is less supporting infrastructure in civilian populations.

Their research efforts are twofold:

- To provide first responders with low-cost simple detection devices, such as single hand-held units and biotickets, to rapidly

determine whether an agent is present, identify it, and gauge its concentration, and

- To develop autonomous, sensitive, low maintenance detection systems that can monitor potential targets.

#### *7.4.5.4 DOE Biological Detection Technology Development Initiatives*

Lawrence Livermore National Laboratory (LLNL) is developing a stand-alone instrument that the researchers envision will provide automated continuous monitoring for many potential biological agents at special events or in high-threat locations. The system includes continuous aerosol sampling, sample preparation, automated fluidic sample handling and transport, detection and identification by flow cytometry immunoassay and nucleic acid recognition (polymerase chain reaction), and automated data analysis and reporting. This system was tested at Pacific Northwest National Laboratory in 1999 to demonstrate its ability to operate a single flow assay autonomously and continuously for over 12 hours and to compare it to a pathogen surrogate.

LLNL also is working in collaboration with Luminex Corporation to develop a capability for using color-coded beads to simultaneously detect and identify multiple pathogens in a single flow cytometry assay.

Sandia and Oak Ridge National Laboratories are developing a self-contained, hand portable system for use in all phases of domestic terrorism scenarios. Using multiple chromatographic separation, each sorting on a different physical property, the researchers hope to be able to provide a unique fingerprint of the agent in the presence of complex backgrounds and thus enhance the ability of achieving a low false alarm rate. Combining this with microfabrication techniques, the researchers believe the Chem lab on a chip will be able to detect a broad range of chemical agents, biotoxins, and viral growth media signatures in a few minutes. They are working on designing a liquid-phase analysis module for the detection of biotoxins and a gas phase analysis module for the detection of chemical agents. In 1999, they demonstrated the ability to transfer this technology to a chip, using etched microchannels. The researchers conducted liquid phase separation of biotoxins in microchannels etched in glass and coated to minimize protein sticking, with laser-induced fluorescence for detection. The first integrated unit is targeted for completion in FY 2000. The researchers plan to upgrade the detection capabilities of this unit by developing additional liquid-phase separation methods, which will add reverse phase, size exclusion and ion-exchange chromatography to increase the likelihood for low false alarm detection of biotoxins.

Oak Ridge also is developing a suitcase size, fieldable quadrupole ion-trap CB mass spectrometer for the U.S. Army. This work builds on the Chem lab on a chip program to try and develop a biological detection technique that allows simultaneous detection and identification of multiple proteins by using MS, thus eliminating the need to separate the biomolecules and then detect them one at a time. The system provides direct atmospheric sampling MS for rapid, real-time detection of airborne bacteria or agents and can be operator-directed or used as a stand-alone monitor. This technology is combined with a capability to analyze collected samples. In 1999, the researchers demonstrated a nanospray interface for direct analysis of proteins and ion-ion chemistry to simplify the resulting mass spectra. Other users of this capability are security, law enforcement, and emergency response officials. The researchers envision that new developments in ion-trap analyzers will increase performance for the next generation biological detector.

Los Alamos Laboratory is using flow cytometry to measure the length of DNA fragments in order to rapidly discriminate among bacterial strains. Researchers noted that they have shown burst sizes from intercalated dyes to provide an accurate measure of the length of DNA fragments, and that the fingerprint of fragment sizes measured in this way allow them to discriminate bacterial species and strains within a species. They stated that advances that they have achieved in speed and sensitivity have enabled fragment distribution analyses that are 100 times faster and 200,000 times more sensitive than the traditional method – pulsed gel electrophoresis. In addition, the researchers claim to have reduced the sample preparation time from 18-24 hours to less than six hours. The scientists are looking for a commercial manufacturer to produce the DNA Fragment Sizing Flow Cytometer.

Lawrence Berkeley National Laboratory is researching the development of biochromic conjugated polymer films that change color from blue to red upon binding a toxin, virus, or bacteria. The sensors are integrated biosensing units that encompass molecular recognition, amplification and signal transduction in one self-assembled microstructure, thus not requiring tagged antibodies, separation steps, or secondary visualization reagents. Their goal is to increase the sensitivity and lower the false alarm rates of these biochromic polymer-based detectors through the use of quasi-orthogonal detection techniques that are incorporated on a single sensor chip to develop a low-cost bioticket.

DOE plans to develop research prototypes of current programs and initiate new programs to research emerging technologies and concepts. Oak Ridge National Laboratory is involved in one such program, the Advanced Multifunctional Biochip, that uses a combination of bioreceptors, on-chip fluorescence detection, and a meso-pump technology to create a miniature, low-cost sensing platform.

### *7.5 Canadian Government Agency Research Efforts*

NBC R&D is conducted at the Canadian Defence Research Establishment Suffield (DRES), a defense science and technology center located in southeast Alberta. Established in 1942, DRES is active in the development of detection systems to provide a warning about the release of and CB weapons that could be used against Canadian troops in their operations. Its mission is to protect the CF against CB warfare agents. DRES's CB detection program is more compact than the U.S., with one agency setting policy. DRES currently employs 141 staff; 60 of which are involved in the CB program. Their entire CB detection program budget is $C2.4M, which is small compared to the funding received by the U.S. military to pursue biological detection technologies.

A central focus of DRES's research is on development of an integrated CBW agent detection system to protect personnel at high value, fixed assets such as headquarters areas, field hospitals and airfields. DRES envisions that, to be effective, the system should consist of a network of remote point detection systems - sentries - capable of autonomous operation, real time detection, and rapid identification of all threat CB agents.

DRES's CB Agent Identification Project is involved in developing detection technologies and analytical techniques for positively identifying CB agents in a variety of contaminated samples. Their goal is to fulfill the CF requirement for an independent chemical, toxin and biological identification and confirmation capability to safeguard the forces in peacekeeping or battlefield operations. To operate effectively in these theatres, the CF requires the ability to identify the exact nature of the CB agent(s) being used.

To support the R&D of the CIBADS, DRES has developed specialized facilities to generate biological aerosols of known characteristics in both chamber and field environments. Work at DRES to develop methods to produce and maintain a desired biological aerosol for reproducible testing has evolved into a sophisticated control system.

DRES has developed two new aerosol test facilities: CWAL (Colin Watson Aerosol Layout) and the Bio-aerosol Test Chamber. The CWAL Field Site features a paved platform that can accommodate four test systems, each co-located with reference sampling equipment. Space is available for four more spots by doubling up at each sampling station.

DRES's bio-aerosol test chamber with an attached sampling facility is equipped with particle concentration control features. Each facility has the equipment to test biological detectors and new collectors. A variety of standard samplers are run simultaneously to obtain reference data. The main chamber unit consists of an aerosol volume, control center and mechanical room. An attached module contains the aerosol sampling facility.

DRES has installed a nitrogen laser light source as part of a remote sensing measurement capability. Light detectors are used to measure fluorescence from biological particles in the chamber. Special optical windows are designed to permit excitation and emission measurements of biological particles confined in the chamber. External optical ports facilitate comparative measurements from a LIDAR system situated outside the chamber. The researchers hope to advance their remote biological detection

capabilities by correlating known aerosol source material with LIDAR signals.

DRES has developed a microchip structure and interconnection assembly for automated immunoassays. For the Automated Microchip Platform For Biochemical Analysis, DRES collaborated with the University of Alberta, Dycor, Alberta Microelectric Corp and Canada West Biosciences. DRDC, DARPA and the National Sciences and Engineering Research Council of Canada (NSERC), jointly funded this project. This project developed an automated microchip-based platform for fabrication of microchip channel networks, and combined the electro-osmotic pumping and capillary electrophoresis for fluid transport and separation. The system enabled the on-chip integration of the key elements in analytical processing: injection, mixing, separation, detection and waste elimination within about three minutes. Normally these steps are done manually and require about 30-60 minutes. Complete on-chip chemical processing for immunoassays of the protein ovalbumin has been carried out. Future work in this project will be to connect the platform to a virtual impactor aerosol collector for automated environmental monitoring and will also be directed towards the design and construction of a multi-channel parallel processor immunoassay device. Assays are being developed that will employ a range of antibody-bases molecular recognition elements including intact monoclonal antibodies, protease-digested antibody fragments and genetically engineered single chain antibodies. DRES is teamed with Dycor for this project. Dycor has performed similar work for DARPA.

### *7.5.1 DRES Biological Detection Technology Development Initiatives*

DRES funded a major R&D project starting in 1993, the CIBADS, to produce a field-portable integrated CB agent detection system for the CF. The CIBADS project called for integration of both CB detection and identification. Canada's goal was to design a full CB spectrum detection in a single sentry system. The aim of this program was to provide maximum protection while minimizing the logistical burden and information integration difficulties of maintaining several separate and independent systems for CB detection.

Since this initial undertaking, DRES has adopted a multi-disciplinary approach to CB warfare agent identification that includes instrumental analytical techniques, immunological methods and other technologies. The principal focus of the R&D is on the development of new techniques for the identification and confirmation of toxins and BW agents. Identification of militarily significant CW agents is secondary to the toxin identification effort, as DRES has an established CW agent identification capability.

DRES invented and was the first to field fluorescent based biological detection, which they have employed in their systems. The Fluorescence Aerodynamic Particle Sizer (now in its second generation, FLAPS 2) is the first aerosol particle sizer that also can measure the intrinsic fluorescence of particles containing living organisms. As a result, it is able to distinguish, in real time, those particles in air which contain living organisms from all other background particles. The performance of the FLAPS is based on the correct selection of target biomolecule fluorescence, the instrument tuning and set-up, and appropriate data analysis and alarming algorithms. DRES licensed the FLAPS technology to TSI for manufacture. TSI has sold many units, including 100 to the U.S. Army.

Using this technology, DRES has developed and deployed the following systems (described in further detail in Section 5):

- The Mobile Atmospheric Sampling and Identification Facility (MASIF)
- Canadian Integrated Biological Agent Detection System (CIBADS)
- CIBADS CB Sentry
- CIBADS II.

The core biological detection technology of CIBADS is the FLAPS 2. According to the Joint Abbreviated Analysis Team, the CIBADS unit performed the best of all candidate technologies at the JFT IV. The particular success of FLAPS over other fluorescence based detection systems is the 355 nm laser used. Other systems use a far more common 266 nm laser. The 266 nm laser is much easier to produce but reportedly has difficulty discriminating between true biological agents and common battlefield contaminants such as diesel exhaust.

Because the Canadian DND leads in the use of fluorescent-based, real-time detection technologies for BW agents, the Canadian R&D program is actively developing advanced models of FLAPS, focusing on resolving military field-use problems with the current, commercially available FLAPS. Researchers noted that although the performance of the technology is great, reduction in size, weight, power and cost are being sought to make fluorescent detection the cornerstone of small, autonomous and affordable BW agent detection systems. Cost is a major impediment to the adoption of this technology, with the cost of a full CIBADS system costing approximately $C400K. This is too expensive for many potential customers, such as states, municipalities and first responders.

DRES currently has $C200K in a royalty product improvement fund and is using these funds with TSI to develop the "Millennium FLAPS". A FLAPS 2 costs approximately $C150K. The goal of the Millennium FLAPS is to produce a system costing approximately $C30K and greatly reduced in size – each system would be about the size of a briefcase. This would allow a significant reduction in the cost and size of a CIBADS-type system. Their R&D program also is focused on real-time technologies that do not employ any consumables or reagents and examines the physical or chemical characteristics of the aerosol particle.

DRES researchers noted that recent advances in biotechnology have opened up new avenues for the preparation of militarily significant quantities of agents in the "mid-spectrum" between classical chemical and classical BW agents. They stated that mid-spectrum agents - including peptide and protein toxins and bioregulators - that scientists long thought to be of limited military use because of production problems a decade ago, have emerged as a real threat. Development of instrumental analytical methods for the identification and confirmation of these threat compounds is now a CF requirement that is addressed by this program. A research goal of the Canadian R&D program is to develop a complimentary technology to the immunoassay system for BW agent identification. The researchers envision that complimentary analytical technologies will be required to provide reliable information to unit commanders and medical personnel. Noting that immunoassays have been one of the most effective technologies used for rapid identification of human etiological agents, advances in molecular biology have led to the real possibility that novel threats undetectable by immunoassay could be developed. Hence, the need for a genetic identification capability for identifying threats where immunoassays or other technologies are limited. This technology would be complementary to other technologies and provide confirmatory data to improve diagnostic accuracy and reduce false identification. To address the needs of rapid identification within CIBADS, clinical diagnostic requirements, and laboratory based unambiguous identification or verification analysis, a genetic analysis method or suite of methods is being implemented.

In addition, a number of instrumental analytical technologies including MS, chromatography, Fourier transform infrared, and super-critical fluid extraction are being targeted as candidate technologies for the identification and confirmation of novel mid-spectrum agents and CW agents. Molecular weight and sequence information, critical to the identification of the toxins, will be determined through the use of liquid chromatography electrospray-MS. When liquid chromatography is employed with tandem MS, DRES researchers anticipate that unambiguous identification of complete unknowns may be possible.

DRES representatives indicated that their current method of identification of BW agents is achieved through the collection of an aerosol sample and analysis using immunochromatographic assays. They believe that this is the most affordable and versatile solution in the near term. Their long-term research involved developing recombinant, engineered antibodies that will increase specificity, selectivity, and producibility of antibodies for BW identification.

One key R&D program is on developing improved antibodies through recombinant DNA technology. DRES researchers indicated that improved antibodies have the potential for both improving immuno-diagnostics as well as immuno-therapy. They believe that recombinant antibodies will have increased sensitivity compared to poly and monoclonal Abs, as well as increased specificity compared to polyclonals.

The researchers also are applying nanotechnology to microfluidics in the hopes of

being able to miniaturize detection devices and instruments. Micro-fabricated devices offer the potential of reducing cost, increasing speed, increasing automation, enhancing integration, and reducing size.

DRES is looking to become involved in international collaborative efforts that will be channeled through TTCP Chemical, Biological and Radiological Defence Group Panel 10 and NATO Panel VII, Sampling and Identification of Chemical Agents, which has toxin identification as its top analytical priority.

## *8.0 CONCLUSIONS*

The conclusions of this study are based on observations of the current technological and business environment associated with biological detection system technologies. The specific conclusions that were drawn as a result of this study are broken out into several general areas: BW agent threat, BW agent detection technology challenges, current systems, R&D, future biological detection system requirements, industrial base, program implementation/fiscal considerations, communications, and testing conclusions. These findings are described in the following sections.

### *8.1 BW Agent Threat*

- The BW agent threat has emerged as one of today's foremost security challenges due to a number of reasons:

  a. The increasing availability and sophistication of biological weapons technology,
  b. The widespread proliferation of ballistic and cruise missiles,
  c. The changing global environment, and
  d. The tremendous lethality of biological agents.

- U.S. and Canadian forces deployed to the Persian Gulf in 1990 found themselves ill-prepared for countering the CB threat. Readiness is only marginally better today.

- BW agents require relatively low levels of scientific and technological support and can be produced using common commercial processes.

- Limited financing and training are needed to establish a BW program.

- BW have low visibility and can be deployed through a rather simple means of delivery.

- CB warfare agents affect humans in different ways. Effects of exposure to chemical agents is almost immediate. But, effects of exposure to biological agents might not be manifest for several days and can affect wider areas because of increased toxicity.

- Both governments are concerned about the potential of terrorists to try to use new, genetically-engineered agents that might escape detection through current detection system capabilities and might defeat conventional methods of treatment.

- Crucial to eliminating or reducing the number of casualties and the spread of contamination is how quickly the release of warfare agents can be detected.

### *8.2 BW Agent Detection Technology Challenges*

- No single sensor detects/identifies all biological agents of interest. Several different technologies may be needed as components of a layered detection network.

- It is difficult to discriminate and measure BW agents from naturally occurring background materials. Real-time detection and measurement of biological agents in the environment is daunting because of the number of potential agents to be identified, the complex nature of the agents themselves, the countless number of similar microorganisms that are a constant presence in the environment and the minute quantities of pathogen that can initiate infection. Potential biological agents can disguise themselves in apparently benign entities.

- Because of the makeup of BW agents, the approaches for detecting these agents differ from those technologies that are employed to detect chemical warfare agents. While BW agents are extremely complex and large in comparison to CW agents, they are only made up of a very limited number of unique building blocks. This means the detection systems have to either:

  a. Exploit the 2- and 3- dimensional configurations of biologics (e.g., using antibodies, gene probes/primers, and possibly chromatography),
  b. Use fairly generic detection/identification technologies like fluorescence, or
  c. Process the supra-molecular BW agents into more manageable sizes to allow generic detection/identification by CW-type technologies (e.g., IMS and MS).

- The lethality of BW agents heightens the requirements for detection system sensitivity, which can lead to increases in cost, size, weight and power requirements with present day technology. On a per-mass basis, BW agents can be billions of times more lethal than CW agents. Hence, the farther the detector is from the agent release line or point, the more sensitive the system must be.

- There continues to be a large gap between the lethal threat aerosol concentration and the limits of detection of current equipment.

### *8.3 Current Systems*

- Biological detection technologies are in a much less mature stage of development than chemical detectors. Most available systems are point detection systems that are either in the field testing stage or still in the laboratory. Stand-off biological agent detection systems are in early stages of development and will not be ready for deployment for several years. Current biological agent detection systems are large, complex, expensive, and subject to false alarms. They can detect only a limited number of biological agents and only after exposure. Sensitivity, selectivity and durability of these detection technologies are not proven.

- Cost is a major impediment to both military and non-military adoption of BW detection systems. However, funding for biological detection systems has been on the rise. Even so, the cost to the military must decrease before military users can create networks of sensors. And, the cost of these systems will need to come down substantially before domestic preparedness operations and commercial users could afford to buy the systems in the quantities that they would require to be effective.

- The small particle size of biological agents requires a complex identification process and detectors. The generic model for a biological point detection system includes a collector, a trigger, a detector, and an identifier.

- Most biological detection systems have significant support requirements, due to the use of wet chemistry and expensive and sensitive reagents. The use of expensive and sensitive reagents is a huge logistics burden on the user. Some currently fielded systems must be manned continuously by specialized personnel and identification depends on having the correct reagents.

- Current biological detection devices/systems require substantial power for operation. Some systems require the use of dedicated generators.

- Current detectors available are stand-alone systems that lack connectivity to military command and control networks. Successful integration of command and control systems with chemical and biological sensors is considered essential for the battlefield.

- No adequate means exist today to detect biological agents within containers or packages non-intrusively or remotely.

- Personnel responding to, managing or investigating a biologically contaminated scene cannot sufficiently detect, characterize, and delimit the extent of hazardous materials in the environment.

### *8.4 R&D*

- The development of BW agent detection and identification systems is one of the most intense research activities in defense R&D.

- Biological detection technologies research emphasis is aimed at:

  a. Improvements to biological detection and identification capability, ideally moving towards detect-to-warn capability,
  b. Emphasis on reduced weight, automation and field-portability,
  c. Integration of components into a single, rugged system that optimizes power while retaining modularity to support upgrades, and
  d. The ability to protect valuable fixed assets such as a field hospital or airfield.

- A number of different candidate technologies are being researched for

possible use in next generation detection systems, dependent upon their ease of use and level of logistical support requirements. Developing dry technologies for these systems would reduce the logistical burden.

- More investment in fast, sensitive and accurate bio-weapon detection is needed.

- Further research into sample collection and processing is required.

- Greater cooperation between military and civil authorities and a closer relationship between U.S. efforts and those of other friendly countries are needed. Military and civil R&D programs conduct R&D in similar areas as well as in support of similar user communities. They pursue many of the same capabilities, target the same types of technologies, and contract with many of the same laboratories to perform the R&D work. However, participation in formal and informal coordination mechanisms has been cited as inconsistent.

- One challenge facing the community is to ensure the effective integration of new and emerging sensor technologies into current and future detection programs. The DoD has used the U.S. programs to focus on (in the near term):

  a. Collector/Concentrators – The goal is to develop a high efficiency, low power consuming collector/concentrator capable of delivering a detectable level from a low concentration aerosol.
  b. Generic Detectors – non-wet chemistry – high performing, small, low power consuming dry detectors are key to ensuring that the military forces do not miss an unorthodox BW agent attack. They also are key to reducing the overall size and logistics burden of the entire detection system.
  c. Stand Off Technologies such as optical stand off technologies like LIDAR, fusing radar signals with an intelligent warning algorithm, improving methodologies for analyzing physical aerosol signatures, miniaturizing and ruggedizing detectors, and exploiting the power of networked systems are needed. There is a big push to examine how to integrate optical stand-off with other technologies.
  d. Reagents – Antibody and gene-based identification systems are the current state-of-the-art but there also is focus on developing reagents for new and emerging threat agents and in exploiting cutting edge molecular engineering techniques to improve the current reagent sets to make them more sensitive, faster reacting and more specific.

### *8.5 Future Biological Detection System Requirements*

- Detection systems need to be deployable and supportable across the entire spectrum of military operations and for the full duration of those operations. Continuous, long term monitoring may be required for high priority fixed sites.

- Systems must have low false-positive rates.

- The ability to detect BW agents in water supplies also is needed. At many of the military's fixed sites, troops draw potable water supplies from uncontrolled civilian sources.

- Power components must be reduced and more efficient power sources (batteries, generators, etc.) developed/integrated into bio-detection systems to reduce the size and weight of the system, to reduce supportability requirements and to increase system utility.

- Desired biological detection features include:

  a. operable with minimal supporting infrastructure
  b. operable in a variety of terrain
  c. must interface with existing and planned command and control systems
  d. robust equipment that can withstand vehicle transport and environmental extremes
  e. man-portable
  f. high-volume automated throughput
  g. inexpensive
  h. disposable or decontamination-capable
  i. minimal requirement for specialized training

j. operable for long periods of time with minimal maintenance
k. long shelf-life
l. broad-ranged and able to add new threat agents rapidly
m. sensitive to civilian population susceptibility
n. low false positive alarm rates that reflect specific mission requirements
o. rapid detection and identification.

- One need of future enhancements to current detection systems is to incorporate technologies that enable better characterization and portrayal of background interference for point and stand-off biosensors.

- Systems capable of non-specific identification, e.g., determining the presence of bacteria, toxins and viruses by targeting generic factors, are highly desirable. Broad based detection may provide a means for detecting biologically engineered threats with signatures that are different from the agents current systems are programmed to identify.

- Improved sample collection systems for air, surfaces, water and soil are needed. DNA based detection/identification is feasible for military field detection requirements only after a sample has been collected, contaminants have been removed from the sample, and a "clean" sample (inhibitors removed) has been presented to the identification component (e.g., PCR, MS). Speed of detection using DNA-based detectors could be accelerated with the development of improved sample preparation systems.

- Troops need handheld sensors that detect airborne BW agents.

- Another needed capability is for non-intrusive detection of biological agents (e.g., screening cargo, mail, packages, etc.).

### *8.6 Technology and Industrial Base*

- The biological agent detection technology industrial base sector is primarily supported by small and medium sized companies.

- Many of these companies are in the development stages of technological maturity, with very small scale manufacturing capabilities.

- Most of the companies involved in this arena have already formed or are actively forming teaming arrangements in order to be able to fulfill requirements.

- Smaller companies are teaming with larger companies, who would act as system integrators in assembling the detection system and make use of flexible manufacturing lines.

- Companies involved in development of technologies for detection systems are not solely focused on biological detection system applications, but rather for use in a variety of commercial applications as well. A number of marketplace factors influence a company's rate of success, including, among others, the company's ability to:

  a. Successfully commercialize a broad range of products
  b. Keep pace with rapidly changing technology
  c. Remain competitive
  d. Fund R&D programs
  e. Manage the patent process
  f. Protect the company's trade secrets
  g. Capitalize on collaborative opportunities and strategic partnerships
  h. Develop products that are in demand in the marketplace
  i. Invest in needed capital equipment/ facilities.

- A number of companies who manufacture laboratory equipment for other markets also are tracking developments in this field, looking at the potential to tailor their technologies and/or instruments for future detection systems.

- CB detection technologies have dual use potential in a number of different fields, including pharmaceutical and medical diagnostics, and monitoring air pollution and air quality in plants, noxious fumes inside enclosed areas, and municipal water supplies.

- The biological detection arena reaps the benefits of advances in other high growth technology areas, including biotechnology, computer technology, display technology, micro electronics, nano technology, communications technology, and low level signal recovery technology.

- The military forces are not the only government entities that have detection system requirements. Detection systems are needed for first responders, the U.S. Secret Service, the Federal Bureau of Investigation, fire departments, airports, embassies, and hospitals.

- There currently is not enough demand for any single biological detection system that would allow companies to make a realistic business case decision on production. Industrial production must be based on dual use technologies because the military is too small a segment of the market.

- Both the U.S. and Canadian military forces have low inventories of some biological detection equipment.

- In the U.S., detection equipment currently fielded would not be adequate to fulfill current Major Theater War requirements.

- The DoD and DND have striven to communicate with industry on their NBC procurement plans for the future through annual APBI (U.S.) and Industry Days (Canada). Both countries' defense departments also are receptive to briefings from industry on their different technologies.

### *8.7 Program Implementation/Fiscal Considerations*

- CB defense efforts of each of the four U.S. Services are coordinated through the CB Defense Program, which has led to a number of joint-Service projects.

- However, each of the U.S. Services also has unique, specific requirements for biological detection systems to meet their needs. Meeting the needs of all Services using common equipment is sometimes difficult, hampering the effectiveness of joint programs. For instance, whereas the USAF can handle a 900-pound detector, the USMC want a detector that weighs just nine pounds. U.S. inter-service disagreements hamper the DoD's efforts to deploy advanced detectors in the field. This has contributed to a lack of preparation in the technology base.

- Canada's research arm for biological detection is centralized at DRES. The U.S. research efforts are more decentralized, more complex, and broader ranging. Many different research components of the U.S. government are involved in U.S. biological detection R&D. Research in this area is conducted by the four Services laboratories, as well as within DOE and DARPA.

- Challenges faced by the DoD and DND are the rapid turnover of promising science and technology products and technologies, shortening acquisition times, and lowering total ownership costs. This necessitates the need to continually track new and emerging technologies and ensure an effective technology transfer/integration process.

- The U.S. funding process is very involved and lengthy, and sometimes hampers the military's ability to move forward with a promising technology or fund a new program. The U.S. players must defend their programs through PPBS process every year. This can cause fluctuations in funding of programs. The Canadian DND has a shorter, more streamlined decision process in which very few decision-makers are involved and, as such, its funding is much more stabilized.

- The U.S. spends more money than Canada to fund a number of different research programs and system development initiatives in the biological detection area. This is a reflection of the size difference between the U.S. and Canadian defense R&D budgets. Given these funding constraints, DND has made considerable progress in technology development.

### *8.8 Communications*

- There are many new players in the biological defense arena, and improvements in communication are needed. Though there is formal and informal program coordination

between the agencies sponsoring R&D, it is inconsistent and does not ensure that potential overlaps, gaps, and opportunities for collaboration are addressed. JPO-BD has cited three challenges:

a. One challenge facing this community is the ability to leverage mission requirements for Domestic, Reserve, and National Guard requirements
b. Another challenge is to overcome the instability of Service requirements
c. A third challenge is to leverage international collaboration.

- Information is lacking on the military forces' operations' prioritized needs, validated CB defense equipment requirements and how programs relate R&D projects to these needs. The requirements process needs to be defined. Competing priorities of a very complex management and oversight bureaucracy can dilute program focus. The DoD is working to alleviate this situation and intends to submit the needed information to Congress in 2001. To accomplish this, the DoD is in the process of developing performance goals and performance measures. These goals and measures will be stated along with the development of the CBDP Strategy Guidance and incorporated into key planning, programming, and budgeting documents. A Performance Plan will be completed during calendar year 2000 and included in the next annual report to Congress. In March, 2000 DND published a revised concept for CF Operations – NBC Defence, and is presently maturing a concept of operations for biological agent detectors.

- DOE and DARPA sponsored programs do not formally utilize user requirements in planning their R&D goals. These government offices have not instituted program performance requirements to measure program performance against desired goals, as required by the Government Performance and Results Act (GPRA).[47] The GPRA required adherence to an overall strategic plan, explicit program goals and measurable performance benchmarks.

- Civilian biological detection domestic preparedness programs lack performance measures and measurable goals. Domestic preparedness needs are not as clearly defined and not specified in as great a detail as the military has defined their requirements. No detailed equipment performance specifications or mission and threat analyses documentation has been prepared. A 1999 GAO report stated that "rapid growth is taking place in the domestic preparedness programs for responding to terrorist attacks and public health initiatives, though no sound threat and risk assessments to establish program requirements and prioritize and focus the nation's investments has been accomplished."[48]

### *8.9 Testing*

- There are insufficient test sites in the U.S. to accommodate all the required testing. In fact, currently there is a backlog of testing of different detection technologies.

- The JFT process is being standardized between primary U.S. and Canadian test facilities. Standard test methodologies, processes and procedures are in place based on previous JFT and the CBR MOU JFT Test and Evaluation Working Group effort. This will allow U.S. and Canadian researchers to compare data based on the same reporting results criteria.

- Additional work must be accomplished in developing and implementing new test methodologies to appropriately test emerging point and stand-off technologies.

### *8.10 Additional Concerns*

- There are different decision-makers involved in determining military and domestic response issues. How to coordinate requirements and program initiatives between these communities and determine what role the DoD and DND should play in civilian biological defense needs is a significant challenge.

- For the U.S., considering that the funding for DARPA and DOE R&D programs have been increasing and combined are projected to be greater than the non-medical R&D

funding for DoD's CBDP for the FYDP, mechanisms for coordination need to be established to ensure that funding is used most effectively, redundant efforts are avoided, and similar requirements are handled jointly.

## *9.0 RECOMMENDATIONS*

The recommendations resulting from this study are designed to overcome the technical, policy, market and testing considerations addressed in the conclusions presented above. The recommendations define specific actions that should be undertaken to foster the advancement of current biological detection system technology and fielding of systems.

Based on the conclusions reached as a result of this analysis into the technology and industrial base for biological detection systems, the NATIBO Biological Detection Technologies Working Group has outlined the following recommendations. These recommendations fall into two categories: those that address technology considerations and those that address policy considerations. These recommendations highlight a roadmap of actions that the U.S. and Canadian governments should embark upon to help ensure that the future biological detection system needs of the military forces are met.

### *9.1 Technology*

- **DoD/DND should target joint R&D and biological detection system programs of mutual interest. Full use should be made of the programs in place in both countries – the U.S. ACTD, the DoD Technology Demonstration Program, the DIR Program, the Canadian Technology Investment Fund, and the Canadian Technology Demonstration Program - to fast track those technologies that demonstrate best value into programs.** By jointly developing biological detection systems, interoperability and supportability can be better ensured. In addition, the military forces can develop and field cutting-edge biological detection capabilities needed now, while pooling scarce resources and ensuring that there are no unnecessary duplicative efforts.

- **Alternative concepts for biological agent detection and active defense should continue to be explored**. At present, there is no silver bullet for universal detection of BW agents. No one method or technique exists today that is capable of detecting all agents. Potential alternatives to currently employed technologies, perhaps discovered through technology breakthroughs achieved as a result of research being conducted in other scientific fields, could advance the capabilities of existing systems. For example, an individual-sized air purification unit based on plasma pyrolysis could be a powerful component of an overall system of active and passive BW defense.

- **DoD/DND should establish funding to support the participation of selected small businesses in their field demonstration of potentially valuable technologies and systems.** Selection criteria would need to be developed to determine what constituted a promising technology. Some promising technologies are being developed by small companies that do not have the internal resources to participate in the JFT.

### *9.2 Policy*

- **Requirements and standards for biological detection systems and how these relate to R&D projects should be better defined.** More detailed information about user needs, CB defense equipment requirements, and how user needs relate to R&D projects may allow more effective coordination to be achieved. If the biological detection community had access to specific data in order to compare the specific goals and objectives of R&D projects, the researchers could better assess whether overlaps, gaps, and opportunities for collaboration exist. Performance measures could also be implemented to help track progress toward goal achievement.

- **A formal process to coordinate areas of research that are supported by multiple agencies and nations should be instated and managed in the U.S. by the Deputy Assistant to the Secretary of Defense for Chemical and Biological Defense.** This coordination process could reduce potential redundant efforts, ensure different agency requirements/concerns are addressed, provide a mechanism to share insights on technology advances/drawbacks, and enhance opportunities for collaboration.

- **The DoD/DND should sponsor bi-annual Biological Detection Conferences.** As demonstrated by the success of the First Joint Conference on Point Detection for Chemical and Biological Defense held in

October, 2000 and the recent DRES CB Industry Day, these types of fora provide an invaluable opportunity for CB communities to share ideas, discuss potential technological advances, and collaborate on possible joint opportunities. Conferences of this nature could help to foster improved dialogue between companies possessing the different pieces of a biological agent detection system as well as with the military organizations. It could prove to be a catalyst to bring electromechanical, optics, electronics, and bio-technology firms together.

- **The JFT process should continue to be supported/funded. Work should continue to improve and standardize test methodologies and procedures as well as develop new methodologies to support emerging technologies.** Improved standards will allow U.S. and Canadian researchers to directly compare data from different testing sites and analyze the effectiveness of different technologies in order to gauge what programs and technologies should be targeted for transition. In fact, it is conceivable that, in the future, with these guidelines, industry could have their technologies tested at different testing sites and their data submitted to the JFT Joint Abbreviated Analysis for analysis. The military Services should take full advantage of the JFTs to objectively evaluate potential technologies for inclusion in BW agent detection systems. These tests provide materiel developers with opportunities to conduct field and chamber testing on their technologies while gaining performance data early on in their programs that they wouldn't otherwise be able to afford. It is an excellent opportunity for them to showcase technologies that have great potential, but lack strong sponsorship. These reports also are open to other appropriate government agencies for their use. The JFT process has been touted as setting the standards for domestic and international biological detection test methodologies. This process has been adopted by Canada and the United Kingdom and set the baseline for JFT VI which was completed in September, 2000 in Canada.

- **A bottom-up review of future biological detection requirements and operational concepts with emphasis on integration, interoperability, and operational utility should be considered.** Earlier research was focused on specific technologies like state-of-the-art power systems, collection systems, and communications and information technologies, but these were carried out without emphasis on the larger system requirements. The current point detection systems all deal with detection of agents after exposure. The next step is medical rather than operational. Future systems should develop a "system of systems" concept that could maintain operational effectiveness in a BW environment.

- **Much more emphasis and sustained, stable funding is needed over a period of time long enough to allow the DoD and DND to research new technologies, move things out of the R&D base, ensure effective command and control communications with other systems, and field them.** Heightened focus and research dollars should be devoted to the biological detection program. There is a clear need for new technologies, especially with the demanding requirements of biological agent detection and identification. Traditional hardware systems and/or immuno-assay approaches may be less effective in dealing with complex environments such as cities and populated areas. And, greater investment in technologies like state-of-the-art power systems, collection systems, and communications and information technology programs for integration into warning and reporting networks is needed. This would allow systems to be reduced in size, be more fully automated and ensure that interoperability requirements are met. Incorporation of these supporting technologies into new/advanced platforms could allow for the use of robotics, unattended ground sensors, and unmanned aerial vehicles. Key to this is ensuring funding stability. A good rapport with industry should be established by stable funding for multi-year programs since industry makes business decisions based on the total level of funding budgeted for that program. In the U.S., program funding reallocations have lead to the disruption of ongoing industrial programs and caused friction with industry partners.

## *Acronyms*

| | |
|---|---|
| ACADA | Automatic Chemical Agent Detector Alarm |
| ACPLA | Agent Containing Aerosol Particle per Liter of Air |
| ACTD | Advanced Concept Technology Demonstration |
| ADM | Advanced Demonstration Model |
| AFM | Atomic Force Microscopy |
| AIDS | Acquired Immune Deficiency Syndrome |
| AMBRI | Australian Membrane and Biotechnology Research Institute |
| APBI | Advance Planning Briefings for Industry |
| APL | Applied Physics Laboratory |
| APS | Aerodynamic Particle Sizing |
| ATD | Advanced Technology Demonstration |
| ATR | Automated Ticket Reader |
| AVS-TLGC | Automated Vapor Sampling-Transfer Line Gas Chromatography |
| BARTS | Biological Agent Real Time Sensor |
| BASIS | Biological Aerosol Sentry and Information System |
| BAWS | Biological Agent Warning Sensor |
| BDG | Bidiffractive Grating |
| BICC | Biological Inertial Collector Concentrator |
| BIDS | Biological Integrated Detection System |
| BIODET | Biological Detection |
| BIRAL | Bristol Industrial Research Associates & Limited |
| BSK | Biological Sampling Kit |
| BW | Biological Warfare |
| CADS | Chemical Agent Detection System |
| CANUKUS | Canada/United Kingdom/United States |
| CB | chemical and biological |
| CBDP | Chemical Biological Detection Program |
| CBMS | Chemical and Biological Mass Spectrometer |
| CBNP | Chemical and Biological Nonproliferation Program |
| CDC | Center for Disease Control |
| CE | Capillary Electrophoresis |
| CEHR | Center for Ecological Health Research |
| CENTCOM | Central Command |
| CF | Canadian Forces |
| CIA | Central Intelligence Agency |
| CIBADS | Canadian Integrated Biological Agent Detection System |
| CID | Collision Induced Dissociation |
| CINC | Commanders in Chief |
| CLIPR | Chemluminescence Imaging Plate Reader |
| $CO_2$ | Carbon Dioxide |
| COTS | Commercial-Off-The-Shelf |
| CPT | Cycling Probe™ Technology |
| CRADA | Cooperative Research and Development Agreement |
| CRLS | Carousel Liquid Sampler |
| CRP | Critical Reagents Program |
| CST | Civil Support Teams |
| CTTS | Counter Terror Technical Support |
| CW | Chemical Warfare |
| CWAL | Colin Watson Aerosol Layout |
| DARPA | Defense Advanced Research Projects Agency |
| DDAP | Domestic Demonstration and Application Program |
| DERA | Defence Engineering Research Agency |

| | |
|---|---|
| DIR | Defence Industrial Research |
| DNA | Deoxyribonucleic Acid |
| DND | Department of National Defence |
| DoD BSK | Department of Defense Biological Sampling Kit |
| DoD | Department of Defense |
| DOE | Department of Energy |
| DRDC | Defence R&D Canada |
| DRES | Defence Research Establishment Suffield |
| DTRA | Defense Threat Reduction Agency |
| ECBC | Edgewood Chemical –Biological Center |
| ECL | Electrochemical Luminescence |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EDT | Engineering Design Test |
| EPA | Environmental Protection Agency |
| ERDEC | Edgewood Research, Development and Engineering Center |
| ESI | Electrospray Ionization |
| EVM | Environmental Vapor Monitor |
| FAB | Fast Atom Bombardment |
| FABS | Force Amplified Biological Sensor |
| FAST | Flow Assay Sensing and Testing |
| FDA | Food and Drug Administration |
| FLAPS | FLuorescence Aerodynamic Particle Sizer |
| FLIPR | Fluorometric Imaging Plate Reader |
| FLOT | Forward Line of Own Troops |
| FOWG | Fiber Optic Wave Guide |
| FPS | Fluorescence Particle Sizing |
| FTIR | Fourier Transform Infrared |
| FTMS | Fourier Transform Mass Spectrometry |
| FY | Fiscal Year |
| GAO | General Accounting Office |
| GC | Gas Chromatography |
| GPRA | Government Performance and Results Act |
| GPS | Global Positioning Systems |
| HHA | Hand Held Immunochromatographic Assay |
| HIV | Human Imnunodeficiency Virus |
| HMMWV | High Mobility Multipurpose Wheeled Vehicle |
| HVAPS | High Volume Aerodynamic Particle Sizer |
| IAT | Institute for Advanced Technology |
| IBAD | Interim Biological Agent Detector |
| ICS | Ion Channel Switch |
| Ig | Immunoglobulins |
| IITRI | Illinois Institute of Technology Research Institute |
| IMS | Ion Mobility Spectrometry |
| IR&D | Independent Research and Development |
| ISO | International Organization for Standardization |
| IT | Idaho Technology |
| JBPDS | Joint Biological Point Detection System |
| JBREWS | Joint Biological Remote/Early Warning System |
| JBSDS | Joint Biological Standoff Detection System |
| JBTDS | Joint Biological Tactical Detection System |
| JCBAWM | Joint Service Chemical/Biological Agent Water Monitor |
| JFT | Joint Field Trial |
| JHU/APL | Johns Hopkins University Applied Physics Laboratory |
| JMCBDS | Joint Modular Chemical and Biological Detection System |
| JPO-BD | Joint Program Office for Biological Detection |
| JSAWM | Joint Service Agent Water Monitor |

| | |
|---|---|
| JSLNBCRS | Joint Service Light Nuclear, Biological and Chemical Reconnaissance System |
| JSMG | Joint Service Materiel Group |
| JSTPCBD | Joint Science and Technology Panel on Chemical and Biological Defense |
| JSWILD | Joint Service Warning and Identification Light Detection and Ranging Detector |
| JTF | Joint Task Force |
| JWARN | Joint Service Warning and Reporting Network |
| Km | Kilometer |
| LAPS | Light Addressable Potentiometric Sensor |
| LCD | Liquid Crystal Display |
| LED | Light Emitting Diode |
| LIDAR | Light Detection and Ranging |
| LIM | Liquid Impingement Module |
| LLNL | Lawrence Livermore National Laboratory |
| LR-BSDS | Long Range Biological Standoff Detection System |
| M | micron |
| MAGIChip | Micro Array of Gel-Immobilized Compound |
| MAGIDU | Mobile Agent Identification Unit |
| MALDI | Matrix Assisted Laser Desorption and Ionization |
| MASIF | Mobile Atmospheric Sampling and Identification Facility |
| MASU | Mobile Atmospheric Sampling Units |
| MBI | Majesco Biologicals, Inc |
| MEMS | Micro-Electromechanics Micro-electronics |
| MIDAS | Micro-fluidic Integrated DNA Analysis System |
| MIPs | Molecularly Imprinted Polymers |
| MIT | Massachusetts Institute of Technology |
| MOU | Memorandum of Understanding |
| MRI | Midwest Research Institute |
| MS | Mass Spectrometry |
| MTW | Major Theater of War |
| NADH | Nicotinamide Adenine Dinucleotide compounds |
| NASA | National Aeronautics and Space Administration |
| NASB | Nucleic Acid Sequence Based Amplification |
| NATIBO | North American Technology and Industrial Base Organization |
| NATO | North Atlantic Treaty Organization |
| NBC | Nuclear, Biological, and Chemical |
| NBCRS | Nuclear, Biological and Chemical Reconnaissance System |
| NDI | Non-Developmental Item |
| NIJ | National Institute of Justice |
| NIST | National Institute of Standards and Technology |
| Nm | Nanometer |
| NMRC | Naval Medical Research Center |
| NQR | Nuclear Quadrupole Resonance |
| NREL | National Renewable Energy Laboratory |
| NRL | Naval Research Laboratory |
| NSERC | National Sciences and Engineering Research Council |
| OCONUS | Outside the Continental United States |
| OMB | Office of Management and Budget |
| P3I | Preplanned Product Improvement |
| PACOM | Pacific Command |
| PBM | Princeton Biological Meditech Corp |
| PCR | Polymerase Chain Reaction |
| PDD | Presidential Decision Directive |
| PDRR | Program Definition and Risk Reduction |
| PPBS | Program, Planning, and Budgeting System |
| PROTECT | Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism |

| | |
|---|---|
| PY-GC-IMS | Pyrolysis-Gas Chromatography-Ion Mobility Spectrometer |
| QDR | Quadrennial Defense Review |
| R&D | Research and Development |
| RAPID | Ruggedized Advanced Pathogen Identification Device |
| RDA | Research, Development and Acquisition |
| RDEC | Research Development and Engineering Center |
| RDTE | Research, Development, Test and Evaluation |
| RF | Radio Frequency |
| RNA | Ribonucleic Acid |
| RTDC | Research and Technology Development Center |
| SASS | Smart Air Sampler System |
| SBCCOM | U.S. Army Soldier and Biological Command |
| SBIR | Small Business Innovative Research |
| SERS | Surface Enhanced Raman Scattering |
| SIMBAD | Sensor Integration and Modeling for Biological Agent Detection |
| SIU | Sample Identification Unit |
| SMART® | Sensitive Membrane Antigen Reaction Test |
| SMO | Semiconducting Metal-Oxide |
| SMSI | Sensors for Medicine and Science |
| SNCP | Sensor Network Command Post |
| SPFC | Single Particle Fluorescent Counter |
| SPR | Surface Plasmon Resonance |
| SR-BSDS | Short Range Biological Standoff Detection System |
| SRI | Stanford Research Institute |
| STTR | Small Business Technology Transfer Program |
| STW | Surface Transverse Wave |
| SUBD | Small Unit Biological Detector |
| TB | Tuberculosis |
| TDP | Technology Demonstration Program |
| TEA | Transversely Excited Atmospheric |
| TIB | Toxic Industrial Biologicals |
| TIC | Toxic Industrial Chemicals |
| TIM | Toxic Industrial Materials |
| TOF | Time-of-Flight |
| TPA | Tripropylamine |
| TSWG | Technical Support Working Group |
| U.S. | United States |
| UAB | University of Alabama |
| UAV | Unmanned Aerial Vehicle |
| UK | United Kingdom |
| UMBC | University of Maryland Baltimore County |
| USAF | U.S. Air Force |
| USAMRIID | U.S. Army Medical Research Institute of Infectious Disease |
| USMC | U.S. Marine Corps |
| UV | Ultra-Violet |
| WMD | Weapons of Mass Destruction |

## End Notes


[1] Seigle, Greg, "U.S. Forces are Poorly Prepared for CB Attacks", *Jane's Defense Weekly*, Sep 30, 1998,

[2] Gebicke, Mark E., Director of Military Operations and Capabilities Issues, "CB Defense: Observations on DoD's Plans to Protect U.S. Forces", before the Senate Committee on Veterans' Affairs. GAO/T-NSIAD-98-83

[3] Inglesby, Thomas, The Germs of War: How Biological Weapons Could Threaten Civilian Populations, *The Washington Post*, Dec 9, 1998

[4] Institute of Spectrochemistry and Applied Spectroscopy - Dortmund and Berlin, http://ims.isas-dortmund.de/ims/ims.html

[5] Clemmer, D.E., M.F. Jarrold, *Journal of Mass Spectrometry*, Vol 32, 577-592 (1997)

[6] ISAS, http://ims.isas-dortmund.de/ims/ims.html

[7] University of Pittsburgh School of Medicine, Department of Pathology, http://path.upmc.edu/cases/case83/gas.html

[8] University of Pittsburgh School of Medicine, Department of Pathology, http://path.upmc.edu/cases/case83/gas.html

[9] Bristol Industrial and Research Associates Limited (BIRAL), Aerosol Science, http://www.biral.com/aerop4.htm

[10] BIRAL http://www.biral.com/aerop5.htm

[11] Imperial Cancer Research Fund, http://www.lif.icnet.uk/axp/facs/davies/flow.html

[12] University of Melbourne Australia, School of Chemistry, Faculty of Science, http://www.chemistry.unimelb.edu.au/MassSpec/homepage/electron_impact.html

[13] University of Melbourne Australia, School of Chemistry, Faculty of Science, http://www.chemistry.unimelb.edu.au/MassSpec/homepage/chemical_ionization.html

[14] University of Melbourne Australia, School of Chemistry, Faculty of Science, http://www.chemistry.unimelb.edu.au/MassSpec/homepage/fab.html

[15] University of Melbourne Australia, School of Chemistry, Faculty of Science, http://www.chemistry.unimelb.edu.au/MassSpec/homepage/electrospray.html

[16] Iowa State University, Mass Spectrometry Laboratory, http://www.public.iastate.edu/~kamel/ms.html

[17] University of Illinois at Urbana-Champaign, School of Chemical Science, Mass Spectrometry Laboratory, http://www.scs.uiuc.edu/~msweb/ion.msms.html

[18] Kuby, J. *Immunology*, W.H. Freeman and Company: New York, 1992

[19] The Antibody Resource Page, http://www.antibodyresource.com/antibody.htm.

[20] Pressman, D. *In Molecular Structure and Biological Specificity, No. 2*, Pauling, L.: Itano, H.A., eds.: American Institute of Biological Sciences: Washington, D.C., 1957; pp 1-17

[21] The Antibody Resource Page, http://www.antibodyresource.com/antibody.html

[22] The Antibody Resource Page, http://www.antibodyresource.com/antibody.html

[23] CB terrorism: R&D to improve civilian medical response/Committee on R&D Needs for Improving Civilian Medical Response to CB Terrorism Incidents, Health Science Policy Program, Institute of Medicine, and Board on Environmental Studies and Toxicology, Commission on Life Sciences National Research Council. http://www.nap.edu.html/terrorism.ch6.html

[24] Altria, Kevin D., Capillary Electrophoresis and Capillary Electrochromatography Webpage, http://www.ceandcec.com/cetheory.htm

[25] Australian Membrane and Biotechnology Research Institute, http://www.ambri.com.au/biosensor_ambri/index_content.html

[26] U.S. Joint Project Office - Biological Defense, http://www.jpobd/net/crp01.htm

[27] Lipsitz, R., "Diagnosis at Home - Pregnancy Tests", *Scientific American* , November 2000, pp110-111

[28] CB Terrorism: R&D to improve civilian medical response/Committee on R&D Needs for Improving Civilian Medical Response to C&B Terrorism Incidents, Health Science Policy Program, Institute of Medicine, and Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. http://www.nap.edu/html/terrorism/ch6.html

[29] U.S. Joint Project Office - Biological Defense, http://www.jpobd/net/crp01.htm

[30] Yin, R., et al, Dendrimer-Based ALERT Ticket: A Novel Hand-Held Nanodevice for Bio Agent Detection, 1st Joint Conference on Point Detection for CB Defense, Oct 2000

[31] CB Terrorism: R&D to improve civilian medical response/Committee on R&D Needs for Improving Civilian Medical Response to CB Terrorism Incidents, Health Science Policy Program, Institute of Medicine, and Board on Environmental Studies and Toxicology. Commission on Life Sciences, National Research Council. http://www.nap.edu/html/terrorism/ch6.html

[32] Affinity Sensors, http://www.affinity-sensors.com/faq.html

[33] STC Technologies Inc, http://www.stctech.com/platform/develop/html

[34] STC Technologies Inc, http://www.stctech.com/platform/develop/html

[35] CB Terrorism: R&D to improve civilian medical response/Committee on R&D Needs for Improving Civilian Medical Response to CB Terrorism Incidents, Health Science Policy Program, Institute of Medicine, and Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. http://www.nap.edu/html/terrorism/ch6.html

[36] Cooper, D.E., et al, Handheld Sensor for Detection of Biological Pathogens Using Upconverting Phosphor Reporters, 1st Joint Conference on Point Detection for CB Detection, Oct 2000

[37] IGEN International Inc., http://www.igen.com/technology.htm

[38] Menking, D.L.,Goode, M.T.,Zulich, A.W.,Myers, E.D., Volker,B.T.,Tanner,R.L., Immunomagnetic One-Step Assay for Detection of Biological Agents, 1st Joint Conference on Point Detection for CB Defense, Oct 2000

[39] Kriz, D., Ramstrom, O., Mosbach, K., *Analytical Chemistry*, 1997, 69, 345A-349A

[40] Betsch, D.F., Access Excellence, The National Health Museum, 1999 http://www.accessexcellence.org/AB/BA/DNA_Fingerprinting_basics.html

[41] CB Terrorism: R&D to improve civilian medical response/Committee on R&D Needs for Improving Civilian Medical Response to CB Terrorism Incidents, Health Science Policy Program, Institute of Medicine, and Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. http://www.nap.edu/html/terrorism/ch6.html

[42] Bloom, M.V., Polymerase Chain Reaction, Access Excellence, The National Health Museum, http://www.accessexcellence.org/RC/CT/polymerase_chain_reaction.html

[43] Kozaryn, Linda, *American Forces Press Service*, Nov 4, 1999, Former Soviets' Bio-War Expert Details Threat

[44] Buley, LTC Don, Advanced Planning Briefing to Industry, September 2000

[45] *Combating Terrorism: Need for Comprehensive Threat and Risk Assessments of Chemical and Biological Attacks*, GAO/NSIAD-99-163, Sep 7, 1999

[46] Erwin, Sandra I., "Chemical/Biological Defense Program Runs Hot and Cold", *National Defense*, Sep 1999

[47] *Combating Terrorism: Need for Comprehensive Threat and Risk Assessments of Chemical and Biological Attacks*, GAO/NSIAD-99-163, Sep 7, 1000

[48] *Combating Terrorism: Need for Comprehensive Threat and Risk Assessments of Chemical and Biological Attacks*, GAO/NSIAD-99-163, Sep 7, 1000

[49] Quan, Cheng and Raymond C. Stevens, "Coupling of an Inducted Fit Enzyme to Polydiacetylene Thin Films: Colorimetric Detection of Glucose", *Advanced Materials,* May 1997

## BIBLIOGRAPHY


Abilek, Ken, 1998, Terrorist and Intelligence Operations: Potential Impact on the U.S. Economy Statement before the Joint Economic Committee, U.S. Congress, May 20, 1998

Affinity Sensors, http://www.affinity-sensors.com/faq.htm

Albert, Roy, U.S. Army Edgewood Chemical Biological Center, Technology Transfer Briefing

Alley, Toney, PM, MAR/NBC, Small Unit Biological Detector Briefing

Alley, Tony, Program Manager's Presentation to Industry on the Joint Warning and Reporting Network

Altman, Wolf P. and Hohe, Donald R., Battelle, JBPDS Block I EMD Program Overview and Summary, presented at 1st Joint Conference on Point Detection, October 23-27, 2000

Altria, Kevin D., Capillary Electrophoresis and Capillary Electrochromatography Webpage, http://www.ceandcec.com/cetheory.htm

American Forces Information Service Defense Viewpoint, prepared Combined Statement before the House Armed Services Committee, Military Research and Development Subcommittee, Defense Research and Development Support to Domestic Emergency Preparedness for Response to Threat of Terrorist Use of Weapons of Mass Destruction Terrorism, March 11, 199

Atlas, R.M., Bioscience, 1999, Combating the Threat of Biowarfare and Bioterrorism: Defending Against Biological Weapons Critical to Global Security

Atlas, RM, 1998, Biological Weapons Pose Challenge for Microbiology Community: Microbiologists Should Help Shape Poli Protecting Against Biological Weapons, ASM News 64

Australian Membrane and Biotechnology Research Institute, http://www.ambri.com.au/biosensor_ambri/index_content.html

Beal, Clifford, Jane's Defense Weekly, November 4, 1998, Facing the Invisible Enemy

Betsch, D.F., Access Excellence, the National Health Museum, 1999, http://www.accessexcellence.org/AB/BA/DNA_Fingerprinting_Basics.html

Biological Warfare and the Implications of Biotechnology, http://www.calpoly.edu/~drjones/biowar-e3.html

Biological Warfare Improved Response Program, 1998 Summary Report on BW Response Template and Response Improvements, March 10, 1999

Bioloigcal Weapons FAQ v.0.43, February 9, 1999, http://www.ocean.ic.net/ftp/doc/disaster/bio/biofaq.html

BIRAL http://www.biral.com/aerop5.html

Bloom, M.V., Polymerase Chain Reaction, Access Excellence, The National Health Museum, http://www.accessexcellence.org/RC/CT/polymerase_chain_reaction.html

Boulet, Dr. Camille A., The Canadian Chemical and Biological Defence Research and Development Program Briefing

Bristol Industrial and Research Associates Limited (BIRAL), Aerosol Science, http://www.biral.com/aerop4.htm

Buley, LTC Don, Advanced Planning Brief to Industry, September 2000

Buley, LTC, Deputy Program Manager for Test and Detection, Biological Detection Test Methodologies Briefing

BW Tech Base (6.2 Programs, Bio Aerosol Trigger/Detector Briefing

Cain, BG Eddie, Biological Defense Briefing, June 22, 1999

Canadian Security Intelligence Service 1998 Public Report, 1999-06-09, Cat No JS71-2/1999 ISBN 0-662-641168-X

Carey, John, Defense News, August 31, 1998, Missile Defense vs. Terror

CB Point Detection Team, U.S. Army Edgewood Chemical and Biological Center, Biological Early Warning Remote, Stand-A Biological Detector Briefing, July 21, 1999

Center for Counterproliferation Research, "The NBC Threat in 2025: Concepts and Strategies for Adversarial Use of Nuclear, Biological and Chemical Weapons, February 1997

Center for Nonproliferation Studies, Chemical and Biological Weapons Resource Page, Federal Structure for Terrorism Respo http://ens.miis.edu/research/ebw/response.htm

Chan, Kwai- Cheung, Director, Special Studies and Evaluations, National Security and International Affairs Division, Observat on Nonmedical Chemical and Biological R&D Programs, htt://www.house.gov/reform/ns/hearings/subfolder/chan3222000.htm

Chemical and Biological Defense Information Analysis Center, State-of-the-Art Report on Biodetection Technologies, July 199

Chemical and Biological Defensive Materiel, International Task Force 32, Memorandum of Understanding Cooperative Prograr The Impact of Biological Warfare on Operations, August 1998

Chemical and biological terrorism : research and development to improve civilian medical response / Committee on R&D Nee Improving Civilian Medical Response to Chemical and Biological Terrorism Incidents, Health Science Policy Program, Institut Medicine, and Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. http://www.nap.edu/html/terrorism/ch6.html

Colton, Rich, Naval Research Laboratory, Immunobead Force Differentiation Sensor

Combating Terrorism: Need for Comprehensive Threat and Rick Assessments of Chemical and Biological Attacks, General Accounting Office, GAO/NSIAD – 99-163, September 7, 1999

Combating Terrorism: Observations on Crosscutting Issues, Statement of Richard Davis, Director, National Security Analysi National Security and International Affairs Division, General Accounting Office, GAO/T-NSIAD-98-164, April 23, 1998

Combating Terrorism: Observations on the Nunn-Lugar-Domenici Domestic Preparedness Program, General Accounting Offi
GAO-T-NSIAD-99-16, October 2, 1998

Combating Terrorism: Status of DoD Efforts to Protect its Forces Overseas, General Accounting Office, GAO/NSIAD-97-254
September 26, 1997

Combating Terrorism: Threat and Risk Assessments Can Help Prioritize and Target Program Investments, General Accounti
Office, GAO/NSIAD-98-74, April 9, 1998

Commission to Assess the Organization of the Federal Government to Combat the Proliferation of Weapons of Mass Destruc
Combating Proliferation of Weapons of Mass Destruction, July 14, 1999

Contamination Avoidance, Navy Website: www.chembiodef.navy.mil/

Cooper, D.E., et al, Handheld Sensor for Detection of Biological Pathogens Using Upconverting Phosphor Reporters, 1st Joint
Conference on Point Detection for Chemical and Biological Detection, Oct 2000.

Counterproliferation Program Review Committee, Report on Activities and Programs for Countering Proliferation and NBC
Terrorism, April 2000

Croddy, Eric, Frost and Sullivan, March 1998, World Markets for Chemical and Biological Warfare Agent Detection

D.E. Clemmer, M.F. Jarrold, Journal of Mass Spectrometry, Vol 32, 577-592 (1997) ISAS, http://ims.isas-
dortmund.de/ims/ims.html

Defence Research and Development Canada, Outline of Program, June 2000

Defence Research Establishment Suffield, R&D Products, http://www.dres.dnd.ca/prooducts/index.html

Defense Evaluation and Research Agency, DERA Annual Report, 1999-2000

Defense News, August 31, 1998, Albright Concerned about Terrorist Threat

Defense News, August 31, 1998, Missile Attacks Could Spark New Anti-Terrorist Tactics

Defense News, March 2, 1998, Canada Spy Agency Warns of Increased Trend in Terrorism

Defense Science Board Task Force, Preserving a Healthy and Competitive U.S. Defense Industry to Ensure our Future Natio
Security Final Briefing, November 2000

Department of Defense Nuclear/Biological/Chemical (NBC) Defense, Annual Report to Congress, March 1999

Department of Defense Nuclear/Biological/Chemical (NBC) Defense, Annual Report to Congress, March 2000

Department of Defense Response, http://www.defenselink.mil/pubs/prolif97/secii.html

Deutch, John; Cater, Ashton, and Zelikow, Philip; John F. Kennedy School of Government, Harvard University, Catastrophic
Terrorism: Elements of a National Policy, 1998

Dickinson, Lt. Col Lansing E., USAF, “The Military Role in Countering Terrorist Use of Weapons of Mass Destruction”, Septe
1999

DRES R&D Bulletin 98002, Development of the Canadian Integrated Bio-Chemical Agent Detection System

Edgewood Chemical Biological Center, Millimeter and Submillimeter Detection Briefing

Edgewood Chemical Biological Center, Peptides as Antibody Substitute and Epitope Mimetics

Eldridge, John, Jane’s NBC Defense Systems, Eleventh Edition, 1998-1999

Emanuel, Dr. Peter A., Edgewood Chemical Biological Center, Reagent Development Briefing

Erwin, Sandra I., National Defense, Chemical, Biological Defense Program Runs ‘Hot and Cold’, September 1999

Experts: U.S. Unprepared for Bio-Terrorists, April 14, 1998, CNN News, http://www.cnn.com/US/9804/14/infectious.threats/

Extracted from Lipsitz, R., *Scientific American* , "Diagnosis at Home - Pregnancy Tests", November 2000, pp 110-111

First Annual Report to the President and the Congress of the Advisory Panel to Assess Domestic Response Capabilities for Terrorism Involving Weapons of Mass Destruction, December 1999, http://www.infowar.com/class_3/00/calss3_tp-terr.shtml

Gebicke, Mark E., Director of Military Operations and Capabilities Issues, before the Senate Committee on Veterans' Affairs, General Accounting Office, Testimony on “Chemical and Biological Defense: Observations on DOD's Plans to Protect U.S. Forces”

Genovese, James A., Edgewood Chemical Biological Center, Disparate Sensor Integration, April 5, 1999

Goode, Michael T., Edgewood CB Center, Gene Based Detection Briefing

Headquarters, Departments of the Army, Navy, and Air Force, FM 3-9, NAVFAC P-467, AFR 355-7, Potential Military Chemical/Biological Agents and Compounds, 12 December 1990

Hearings on National Defense Authorization Act for Fiscal Year 2000 – H.R. 1401 and Oversight of Previously authorized Programs Before the Committee on National Security, House of Representatives, Military Research and Development Subcommittee on Title II – Research, Development, Test, and Evaluation, March 11, 1999

History of Chemical and Biological Detectors, Alarms and Warning Systems, U.S. Army Soldier and Biological Command

IGEN international Inc., http://www.igen.com/technology.htm

Imperial Cancer Research Fund, http://www.lif.icnet.uk/axp/facs/davies/flow.html

Inglesby, Thomas, The Washington Post, December 9, 1998, The Germs of War: How Biological Weapons Could Threaten Civilian Populations

Institute of Spectrochemistry and Applied Spectroscopy – Dortmund and Berlin, http://ims.isas-dortmund.de/ims/ims.html

Iowa State University, Mass Spectrometry Laboratory, http://www.public.iastate.edu/~kamel/ms.html

Izzo, COL Leonard, Future Operational Capabilities Update, June 22, 1999

Jensen, Janet, Joint Service Agent Water Monitor, U.S. Army Soldier and Biological Chemical Command

Joint Program Office for Biological Defense Assessment of Biological Warfare Detection, Joint Program Office for Biological Defense, September 13, 1999

Joint Project Office - Biological Defense, http://www.jpobd.net/crp01.htm

Joint Service Chemical and Biological Defense Program Overview, FY 98-FY99

Joint Service NBC Defense Modernization Plan, March 1999

Joint Warfighter Science and Technology Plan, Chapter IV – Achieving Joint Warfighting Capability Objectives

Kozaryn, Linda, American Forces Press Service, November 4, 1999, Former Soviets' Bio-War Expert Details Threat

Kriz, D., Ramstrom, O., Mosbach, K., *Analytical Chemistry*, 1997, 69, 345A-349A.

Kuby, J. Immunology: W.H. Freeman and Company: New York, 1992.

Lederer, Melissa, Naval Surface Warfare Center, Chemical and Biological Defense Equipment and Naval Ships, Nuclear Biolc and Chemical Symposium, June 22, 1999

Loeb, Vernon, The Washington Post, January 23, 1999, Embassy Attacks Thwarted, U.S. Says

Long, Dr. Randolph, Edgewood Chemical Biological Center, Biodetection The Base Briefing

Malatesta, Mark, Detection Project Officer, Program Director for Bio Defense Systems, Technology Assessment for the Joint Biological Standoff Detection System

Menking, D.L., Goode, M.T., Zulich, A.W., Myers, E.D., Voelker, B.T., Tanner, R.L., Immunomagnetic One-Step Assay for Detection of Biological Agents, 1st Joint Conference on Point Detection for Chemical and Biological Defense, Oct 2000.

Miller, Judith and Broad, William, New York Times, January 22, 1999, Clinton Describes Terrorism Threat for 21st Century

Mulholland, David, Defense News, April 20-26, 1998, Chem-Bio Attack Fears Drive Detection Device Market

Mulholland, David, Defense News, July 26, 1999, Vast Biological Research Tops Pentagon List of Urgencies

Mulholland, David, Defense News, June 22, 1998, U.S. Experts Urge New Tactics Against Bio-War Threat

Office of the Inspector General, Department of Defense, Audit Report, The Low-Rate Initial Production Decision for the Joint Biological Point Detection System, September 11, 2000

Office of the Secretary of Defense, "Proliferation: Threat and Response", 3rd edition, January 2001

Okie, Susan, The Washington Post, February 23, 1999, Preparing for Invisible Killers.

Olsyzk, Rudy, MARCORSYSCOM, Program Overview for the Joint Service Light NBC Reconnaissance System

Parachini, John V., Center for Nonproliferation Studies, Monterey Institute of International Studies, Statement before the Hous Subcommittee on National Security, Veterans Affairs, and International Relations, “Combating Terrorism: Assessing Threats Risk Management, and Establishing Priorities”, July 26, 2000

Paterno, Dorothea and Loerop, William, Edgewood Chemical Biological Center, Ambient Background Characterization

Phelps, Kirkman R., SBCCCOM, Contamination Avoidance Overview for the Joint Service IPT for Industrial Base, April 27, 19

Pincus, Walter, The Washington Post, February 26, 1999, U.S. Preparedness Faulted

Potomac Institute for Policy Studies, Seminar on Emerging Threats of Biological Terrorism: Recent Developments, Proceedi Report, June 16, 1998

Potomac Institute, Biological Terrorism Study Summary, http://www.potomacinstitute.com/projects/cbt/summary.htm

Pressman, D. In Molecular Structure and Biological Specificity, No. 2: Pauling, L.: Itano, H.A., eds.: American Institute of Biological Sciences: Washing, D.C., 1957; pp 1-17.

Proliferation Threat, January 3, 1998, http://www.voa.gov/editorials/07590.htm

Pugliese, David, Defense News, April 26, 1999, Canada Spy Agency Warns of Increased Trend in Terrorism

Pugliese, David, Defense News, Commerce Outweigh Terrorist Precaution on U.S-Canada Border

RDT&E Budget Item Justification Sheets, February 2000

Reeves, Col Stephen V., Next Generation NBC Reconnaissance Systems and Army Transformation, presented at the CB Advance Planning Briefing for Industry, September 20, 2000

Resnick, Dr. I. Gary, Defense Threat Reduction Agency, The DoD Chemical/Biological Defense Science and Technology Pro September 19, 2000

Roberts, Brad, Institute for Defense Analyses, Biological Weapons in Major Theater War, November 1998

SBCCOM Industrial Base Planning Office, Supplement to “Status of Companies/Industries and Suppliers of NBC/Smoke Sec December 1998

Seigle, Greg, Jane’s Defense Weekly, Sept. 30, 1998, “U.S. Forces are Poorly Prepared for Chemical and Biological Attacks

Sheridan, Brian, Statement by Brian Sheridan, Assistant Secretary of Defense for Special Operations and Low-Intensity Cont Before the Subcommittee on Emerging Threats and Capabilities of the Committee on Armed Services, March 24, 2000

Smardzewski, Dr. Richard R. Integrated Biodetection Advanced Technology Demonstration FY 96-00, Briefing to TARA Pane

Snyder, Peter A., Field Analytical Chemistry and Technology, Chemical and Biological Aerosol Detection nand Identification Field Analytical Instrumentation, Volume 3, Issues 4-5, 1999

Spencer, Carmen, Director, CB Defense Directorate, Defense Threat Reduction Agency, Defense Threat Reduction Agency Overview Briefing

STC Technologies Inc, http://www.stctech.com/platform/develp.html

Stern, Leonard, Ottawa Citizen, In the Battle Against Bioterrorism, Canadian Researchers Are on the Front Line, September 1999

Sullivan, B.M., Evans, B.W., and Allen, P.W., Systems and Information Technology Journal Review, Spring/Summer 20000, Volume 8, Number 1, Biological and Chemical Warfare Defense Sensors and Systems

TechBriefs, Army Research Laboratory, “Advantages of Biotechnology, Nanomaterials are Combined in New Technology Prog May 2000

The Antibody Resource Page, http://www.antibodyresource.com/antibody.html

The Health of Chemical-Biological Defense in the U.S. Military, A White Paper by the NBC Industry Group, November 1997, http://www.nbcindustrygroup.com/white/htm

The Non-Medical Interagency Working Group, Chemical, Biological and Radiological Combating Terrorism Research and Development Report, August 1999

U.S. Department of Energy, Chemical and Biological Nonproliferation Program, CBNP Strategic Plan, March 28, 2000, http://www.nn.doe.gov/cbnp/foreword.htm

University of Illinois at Urbana-Champaign, School of Chemical Science, Mass Spectrometry Laboratory, http://www.scs.uiuc.edu/~msweb/ion.msms.html

University of Melbourne Australia, School of Chemistry, Faculty of Science, http://www.chemistry.unimelb.edu.au/MassSpec/homepage/chemical_ionization.html

University of Melbourne Australia, School of Chemistry, Faculty of Science, http://www.chemistry.unimelb.edu.au/MassSpec/homepage/electron_impact.html

University of Melbourne Australia, School of Chemistry, Faculty of Science,
http://www.chemistry.unimelb.edu.au/MassSpec/homepage/electrospray.html

University of Melbourne Australia, School of Chemistry, Faculty of Science,
http://www.chemistry.unimelb.edu.au/MassSpec/homepage/fab.html

University of Pittsburg School of Medicine, Department of Pathology, http://path.upmc.edu/cases/case83/gas.html

University of Pittsburg School of Medicine, Department of Pathology, http://path.upmc.edu/cases/case83/gas.html

US Joint Project Office - Biological Defense, http://www.jpobd.net/crp01.html

Wade, COL John, Acting Deputy Assistant to the Secretary of Defense for Counterproliferation and Chemical/Biological Defe
Advance Planning Briefing for Industry, June 22, 1999

White House Fact Sheet on New Efforts to Combat Terrorism, President Clinton Unveils New Efforts to Combat Terrorism, Ma
15, 1999

Wong, Ngai, Disparate Sensor Integration/Information Management

Yin, R., et al, Dendrimer-Based ALERT Ticket: A Novel Hand-Held Nanodevice for Bio Agent Detection, 1st Joint Conference o
Point Detection for Chemical and Biological Defense, Oct 2000.

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***Affymetrix***
***Santa Clara, California***

### *Company Overview*

Affymetrix is developing state-of-the-art technology for acquiring, analyzing and managing complex genetic information for use in biomedical research, genomics and clinical diagnostics. Their goal is to capitalize on their work in the DNA probe array field by applying their GeneChip® technology to three primary areas: gene expression monitoring, polymorphism analysis and disease management. The company began independent operations in Santa Clara, California in 1993 and in 1999 added a second manufacturing facility in West Sacramento, California.

Affymetrix has developed and intends to establish its GeneChip® system as a platform for acquiring, analyzing and managing complex genetic information in order to improve the diagnosis, monitoring and treatment of disease. The company's GeneChip® system consists of disposable DNA probe arrays containing gene sequences on a chip, reagents for use with the probe arrays, a scanner and other instruments to process the probe arrays and software to analyze and manage genetic information. Affymetrix's GeneChip® technology can be used for nucleic acid analysis applications including sequence analysis, genotyping and gene expression monitoring. Commercial sales of their GeneChip® system for research use began in 1996. Their customers are pharmaceutical and biotechnology companies, academic research centers and clinical reference laboratories primarily in the U.S. and Europe. Affymetrix now holds more than 70 issued U.S. patents and approximately 275 pending patent applications.

Affymetrics is in the early stages of development and commercialization to their technologies and they have just begun to incorporate their technologies into commercialized products. The company's GeneChip® system has thus far been sold solely for research use, and the majority of these sales have been for their expression-monitoring application.

Affymetrix intends to manufacture its disposable DNA probe arrays, arrayers and scanners, fluidics stations and software in-house and contract with third party suppliers to manufacture certain GeneChip® scanners, hybridization ovens and reagents for its GeneChip® system. At present, they can produce more than 10,000 wafers annually.

For the quarter ended March 31, 2000, Affymetrix's revenue increased 104% to $40.2M, up from revenue of $19.7M for the quarter ended March 31, 1999. Product sales increased 115% to $36.7M for the quarter ended March 31, 2000 up from $17.1M in the comparable period of 1999.

Affymetrix, Inc. acquired Genetic MicroSystems, Inc., a privately-held DNA instrumentation company located in Woburn, Massachusetts, in 1999. Affymetrix anticipates that this company's spotted arrays will complement their GeneChip® gene expression product line, leading to development of a more comprehensive set of array solutions.

InphoGene BioCom Inc., a private company, intends to use Affymetrix's GeneChip® probe arrays to build an Internet-based commercial reference source for gene expression information relating to cardiopulmonary disease (heart, blood vessel and lung).

### *Technology Development*

Affymetrix continues its research into expression monitoring, polymorphism analysis and disease management fields. The company is focused on four types of research: basic research to explore and expand the potential uses of DNA probe arrays and to discover new technologies; applied research aimed at generating polymorphism databases and products; core technology development, such as the design of fully integrated systems for complex genetic information management; and novel manufacturing methods to improve the efficiency of the company's probe array production processes.

Affymetrix's GeneChip® technology uses miniaturized, high-density arrays of oligonucleotide probes to analyze genetic information. It consists of application-specific oligonucleotide arrays, instruments to process and analyze the arrays, and bioinformatics tools to manage and mine the data.

The company's GeneChip® probe arrays are created and utilized by first defining the set of

oligonucleotide probes to be synthesized. With this information, computer algorithms are used to design photolithographic masks for use in manufacturing the probe arrays.

Probe arrays are then manufactured by Affymetrix's proprietary, light-directed chemical synthesis process, which combines solid-phase chemical synthesis with photolithographic fabrication techniques employed in the semiconductor industry. Using a series of photolithographic masks to define chip exposure sites, followed by specific chemical synthesis steps, the process constructs high-density arrays of oligonucleotides, with each probe in a predefined position in the array. Multiple probe arrays are synthesized simultaneously on a large glass wafer. This parallel process enhances reproducibility and helps achieve economies of scale. The wafers are then diced, and individual probe arrays are packaged in injection-molded plastic cartridges, which protect them from the environment and serve as chambers for hybridization.

Once fabricated, the GeneChip® probe arrays are ready for hybridization. The nucleic acid to be analyzed - the target - is isolated, amplified and labeled with a fluorescent reporter group. The labeled target is then incubated with the array using the fluidics station. After the hybridization reaction is complete, the array is inserted into the scanner, where patterns of hybridization are detected. The hybridization data are collected as light emitted from the fluorescent reporter groups already incorporated into the target, which is now bound to the probe array.

Probes that perfectly match the target generally produce stronger signals than those that have mismatches. Since the sequence and position of each probe on the array are known, by complementarity, the identity of the target nucleic acid applied to the probe array can be determined. The GeneChip® probe array portfolio includes human, rat, urine, yeast and *E. coli* genome expression analysis arrays; Rat Neurobiology, Rat Toxicology and Human Cancer Biology directed expression analysis arrays; as well as custom expression analysis arrays, the GenFlex Tag array, genotyping products and disease management products.

The company's R&D efforts have been supported in part by government grants, including a 1994, $31.5M grant for the U.S. Department of Commerce Advanced Technology Program. Together with Molecular Dynamics, a wholly-owned subsidiary of Amersham Pharmacia Biotech, they developed a miniaturized DNA diagnostic device. The grant provided for the development of an advanced miniaturized nucleic acid diagnostic device intended to reduce the costs and increase the speed and reliability of DNA analysis. The company developed a prototype of the device and is pursuing further development.

Affymetrix introduced the GeneChip® HuSNP Mapping Assay, a tool for conducting genetic linkage studies. This is a first in a planned series of Affymetrix high-density genotyping products. They intend to offer a new line of GenFlex Tag arrays to enable researchers to develop their own custom assays. These arrays will be initially targeted to fine mapping and association studies aimed at pinpoint disease susceptibility genes and predicting drug response.

The company is also working in conjunction with Roche Molecular Systems, Inc. and bioMerieux, Inc. to develop products and next generation instrumentation that will incorporate advances from Affymetrix's expression monitoring and genotyping programs to create new tools for therapies for individual patients. These products could be used to collect information on patients from the time of diagnosis to the end of therapy and measure the outcomes of various treatment protocols. These new GeneChip® disease management products could also be used for bacterial/virological testing and water quality monitoring.

***Battelle***
***Columbus, Ohio***

### *Company Overview*

Battelle is a billion-dollar company that develops, manages, and commercializes technology. Headquartered in Columbus, Ohio, Battelle has a worldwide staff of 7,500 scientists, engineers, technicians, and supporting specialists. Each year, Battelle's business operations conduct thousands of programs for some 2,000 companies and government agencies. Typically, this work results in 50 to 100 patented inventions each year. Battelle-owned buildings and equipment are valued at more than $518 million.

Battelle's focus is on developing high-quality products and reducing time-to-market for its clients. Battelle developers insert technology into systems and processes for manufacturers; pharmaceutical and agrochemical industries; trade associations; and government agencies supporting energy, the environment, health, national security, and transportation. Following are some examples of Battelle technologies:

- For the U.S. Army and the U.S. Navy, Battelle developed electronic "dog-tags" with embedded computer chips containing medical and personnel data. Battelle developers then transferred this technology to the commercial sector for use in "smart cards," which incorporate computer chips that store sizable amounts of data, such as medical records or highway toll transactions, in a device the size of a credit card.

- Battelle scientists developed a technique called digital optical recording that serves as the critical design element for compact discs and disc players.

- Battelle scientists played a crucial role in developing the office copier machine (xerography process). In all, Battelle was awarded more than 250 patents related to the dry-copying process.

- Battelle conducted studies for the U.S. Treasury that ultimately led to the adoption of the "sandwich" coin now used by the Mint.

- Battelle evaluated candidate symbols and selected the bar code symbol that now enables automated check-out and inventory control at super-markets and other stores. Battelle has also invented an invisible bar code for specialized applications.

- Battelle helped the U.S. Postal Service develop postage stamps with moisture-activated glues that have bonding strengths capable of withstanding the rigors of automated mail processing.

In addition to headquarters in Columbus, Ohio, Battelle has major technology centers in Richland, Washington, where the company manages the Department of Energy's (DOE's) Pacific Northwest National Laboratory; Long Island, New York, where Battelle partners with the Research Foundation of the State of New York in managing Brookhaven National Laboratory; Golden, Colorado, where Battelle partners with Midwest Research Institute in managing the National Renewable Energy Laboratory; Oak Ridge, Tennessee, where they partner with the University of Tennessee in managing the Oak Ridge National Laboratory; and Geneva, Switzerland. Specialized facilities, regional centers, and offices are located in 66 other cities in the United States and worldwide.

Battelle has been a major player in the development of biological detection systems. This capability and experience encompasses basic biological phenomenology, system engineering, aerosol sciences, testing methodology, manufacturing and program management as well as the support disciplines.

In Battelle's Government Market Sector, Battelle works with the DoD and operational commands to advance the state of the art of technology in CB defense. Specialties include defining functional requirements, assessing operational concepts, performing independent test and evaluation studies/analyses, and developing engineering prototype systems for specific applications. Company researchers work in this arena entails computer and process engineering, analytical laboratory experimentation, chemical analyses instrumentation, hazardous materials testing, and design/manufacturing. Battelle supports the DoD in contamination avoidance, individual protection, test and evaluation, and demilitarization/environmental restoration.

Battelle's chemical defense work with the DoD has expanded to develop and demonstrate technologies for destroying chemical weapon stockpiles in the Former Soviet Union.

### *Technology Development*

Since Operation Desert Storm identified shortfalls in biodetection within the U.S. military and its Allies, Battelle has been supporting the DoD and other organizations in the design, development, manufacture, testing and support of several biological detection technologies and systems. Examples of these efforts are summarized below:

Biological Integrated Detection System (BIDS): Working with the U.S. Army, Battelle developed the conceptual design and architecture of the BIDS, resulting in the BIDS Conceptual Formulation/Design. This concept formulation included a market survey of COTS and developmental technologies that could meet the BIDS detection requirements in the near term as well as projections of future capabilities. The system architecture developed during this project still is the basis of current and future biological detection systems. This study, completed in 1993, subsequently lead to the development of the BIDS NDI system, type classified in 1995 and subsequently to the BIDS P3I (Pre-Planned Product Improvement) system. This latter system introduced technology improvements by replacing older COTS equipment with more state-of-the-art equipment developed by the military.

As part of the BIDS program support, Battelle performed several projects including:

- Large volume production of custom reagents for operational use by the BIDS
- Aerosol collector/concentrator enhancements as well as integration of the collector with the flow cytometer detector
- Integration of a commercial particle sizer with a high-volume air-to-air concentrator, and relevant software and detection algorithmic modifications, to act as an effective trigger device
- Updates to the market survey as to next generation technologies applicable for further enhancements of the BIDS performance.

Small Unit Biological Detector (SUBD): For the USMC, Battelle is developing the SUBD – the first automated, integrated, battery-operated biological detection system for use by the CBIRF. Phase I, conducted in 1995, was a quick reaction effort that demonstrated the ability to develop a user-friendly, lightweight (~130 lbs.) unit that combined a particle counter trigger, aerosol collector and identifier with a computer to automate the entire process of biological detection. The effort from design initiation to delivery of the demonstrator to Camp Lejuene was approximately ten months in duration. To achieve the battery operation, Battelle developed two reduced power technologies:

- Biological Inertial Collector Concentrator (BICC) – Using a rotating array of wire filaments, the BICC demonstrated comparable performance to high power collectors (i.e. >500 Watts) but requiring only 50 Watts.
- Biorefractometer – This device uses an integrated optic device that is coated with antibody stripes for each analyte of interest allowing for the simultaneous identification of biological warfare agents of interest. The response time of this device is 15 minutes with detection sensitivities comparable to those requiring longer processing times.

As part of the system design, Battelle evaluated several low power collector/concentrators as to their ability to concentrate an aerosol sample for analysis by an identifier vs. particle size. This evaluation utilized the High Volume Aerosol Delivery System and its testing methodology developed at Battelle.

Battelle's current Phase 2 efforts on the SUBD program focus on the identifier component development. The identifier is based on the bidiffractive grating (BDG) technology – a variant of the Biorefractometer. The BDG is an evanescent wave sensor that produces a measurable signal change when biological agents are captured by the sensor; the signal is captured by the optical read head and analyzed for response. The identifier itself is an integration of a number of disciplines including optics, chemistry, fluidics, and mechanical, electrical, and software engineering.

Selection of SUBD technologies is expected to be made in early 2001. Integration of the prototype system will ensue to combine the

identifier, biological aerosol collector, and trigger/ detector into the system. Fluidics, system control software, and other assembly elements necessary for system operation will be integrated also at that time.

Automated Ticket Reader: The current standard for screening field samples is immunochromatographic assays, also known as HHA. These devices are typically visually read after a 15-minute "development" period. For the Federal Bureau of Investigation and Department of State, Battelle developed a battery-operated, automated handheld ticket reader to remove operator subjectivity in assessing the positive or negative results from the HHA. This device also has the potential to provide a quantitative assessment of the collected sample as well as improving the sensitivity of the HHA performance.

Joint Biological Point Detection System (JBPDS): Beginning in 1997, the JBPDS Engineering and Manufacturing Development program developed a common biological detection capability for all four U.S. military services. This system provides an automated, integrated biological detection system for deployment in shelters, around airbases and naval ports, on board ships and by forward fighting units. By providing a common set of components, logistical support savings is accomplished via the use of a common set of hardware that is interchangeable among the services as well as common consumables. A major objective of the system design was to totally automate the biological detection process.

During Phase 1 of engineering and manufacturing development, Battelle was responsible for designing, integrating, and testing the Biosuite; in addition to the hardware development side of the program, Battelle was responsible for the Biosuite software development that included algorithm development. In addition to performing complete EMD on these components, Battelle led an integrated process team to select the technologies to be developed, planned the biological testing of the systems, and conducted many of the tests in their facilities. Battelle was also the lead for MANPRINT and System Safety analysis of the overall system as well as performing the Bio-Performance, environmental and human factors engineering testing. Battelle manufactured 28 Biosuite systems for use in integration and testing, including Government lead developmental and operational testing.

During this Phase 2 effort, Battelle increased its responsibility for all the hardware components. This included upgrading the hardware components received from the Government to permit the continuation of the Engineering Design Tests (EDT). Battelle completed EDT and documented the results. Battelle also supported the software integration. Battelle conducted functional configuration audit and regression physical configuration audit on the final system design. Finally, Battelle delivered the hardware, supported its installation and provided support during Government testing, production qualification test/operational assessment.

In October, 2000, Battelle was awarded a firm-fixed-price subcontract to build the major components for nine Joint Biological Point Detection Systems Biosuites as well as perform the system engineering and integration of these systems.

Biodetection Component/System Testing: Incipient with those efforts listed above, Battelle has invested in facilities and procedures that allow the testing of components and systems as to their biological detection performance. These include several BL-2 and BL-3 safety facilities for use of live biological agents. Additionally, Battelle has developed the expertise and know-how to reproducibly and controllably challenge components and systems. Innovative alternative testing methodologies have been developed to increase test efficiency and yield robust test scenarios that lead to statistical data to determine the probability of detection and false alarm rates. Currently, Battelle is developing improved equipment that will allow the testing of large systems (e.g. shelter mounted biological detection systems) against ambient aerosol backgrounds while simultaneously being controllably challenged by simulants. After characterizing this equipment, it will be provided as an asset to Government; being a transportable resource, this system can be moved to any location to test systems against various ambient aerosol loadings.

***BioDTX***
***Palo Alto, California***

### *Company Overview*

BioDTX is located in Palo Alto, CA, and has offices in Washington, D.C. The Company has acquired the exclusive license to polydiacetylene lipid based identification technologies from Lawrence Berkeley National Laboratory. This technology can be used for pathogen identification and for predictive medicine uses in a lab, medical office, the home or in a field environment to instantly identify pathogens as well as to identify optimal drugs for unknown pathogens. For example, targeting respiratory pathogens, a simple Kleenex® like tissue can have an active component printed on it to identify key pathogens.

### *Technology Development*

BioDTX's identification technology is based on the unique ability of polymerized lipid polydiacetylenes to change color in response to molecular stress. To construct a sensor capable of detecting biological pathogens, a ligand, or a mixture of ligands, known to bind to the pathogen are mixed into or synthetically coupled to a lipid carrier and are polymerized into a polydiacetylene thin film. When the target pathogen binds to the ligands, the molecular stress directly disrupts the order in the polymer film and causes the polydiacetylene film to turn from blue to red[49]. This color change happens very rapidly, usually in seconds. The ligands used are specific to the pathogens and avoid the common cross reactivity problems of antibody based methods.

BioDTX can use standard offset, high speed and ink-jet printers to apply a lipid polydiacetylene based detection fluid to plastic films, paper and most solid surfaces, e.g. glass, or a facial tissue. These sensors could be made in the form of a badge for a dosimeter type of exposure measurement, or a liquid kit for rapidly sampling an exposed area. A person with suspected exposure to a pathogen could open a package of special tissues, blow his/her nose into the tissue then simply look for the characteristic alternating red-blue pattern to emerge. If this pattern develops, the individual will know he/she has been exposed to the pathogen and should then follow instructions on the package applicable to the pattern.

This is an extremely low cost, robust, and simple screening device that could be used to initiate early treatment in exposed individuals. The color change that identifies a specific pathogen can also be arranged in pattern to identify the appropriate drug or antidote for the targeted pathogen and to provide an easy self-diagnosis capability. It currently takes as little as one second after exposure for the concentration of a particular toxin to develop to the point where there is a sufficient sample to cause a color change to occur on a tissue. Specific development times vary as a function of the architecture of the particular sensor and the characteristics of the pathogen.

### *Biopraxis, Inc.*
### *San Diego, California*

#### *Company Overview*

San Diego-based Biopraxis was established as a woman-owned small business in July, 1995 to position the company to commercialize its biotechnologies through Government and private customer contracts and collaborations with strategic teaming partners. Since winning its first contract in April, 1996, Biopraxis has grown from a staff of two in a 1,200 square foot office suite to a well-equipped 9,400 square foot R&D facility, and supports scientists at collaborating organizations as well. To date, there has been no outside venture capital investment.

Biopraxis has relied on government R&D and product development contracts, especially those established for 'high-risk/high-reward' technologies, as the initial source of funding for its technologies. Biopraxis is developing its three technologies under contracts for the DARPA, the U.S. Army Research Office, the U.S. Army SBCCOM, NASA, the Office of Naval Research, the Department of Commerce, the U.S. Army Medical Research and Materiel Command, the DOE, the Naval Surface Weapons Center (Carderock), the Maritime Technology Advanced Shipbuilding Enterprise, the Water Environment Research Foundation, and the California Office of Strategic Technology, with several additional proposals selected for award.

Biopraxis' solid-state biochip technology, the CB-Sherlock technology, is being developed for the detection and specific identification of chemicals and micororganisms. Company representatives have stated that this technology has been shown to be capable of identifying pathogens at the subspecies level, differentiating between viable and nonviable cells, and differentiating between cells of exactly the same strain grown under conditions that induce or suppress the expression of virulence factors. They have also stated that it has been shown to be capable of detecting inorganics and organics simultaneously; of detecting very low-weight as well as high-weight molecules; and of differentiating among sample constituents that cross-react with biomolecules such as antibodies.

Their technology is protected by intellectual property rights consisting of patents issued, patents pending, invention disclosures, and trade secrets. Biopraxis' first patent on the biochip technology was issued in February, 1999, and its second in March, 2000. International applications on these omnibus patents, which cover the underlying processes as well as any devices based on those processes, have been filed. Additional domestic and international patents targeting additional features of the original invention, especially those which position the technology for high-priority applications in the pharmaceutical, life sciences research, and drug discovery areas, are being pursued. The company also has licensing rights to the suite of software being used in the development of CB-Sherlock products.

#### *Technology Development*

CB-Sherlock is a solid-state biochip technology for detecting multiple targets, chemical as well as microbial, in complex samples, with little or no sample preparation. Company representatives point out that it has the potential to detect targets ranging from CW agents and low-molecular-weight nonpeptide toxins, to protein toxins, to bacteria, spores, and viruses - simultaneously. Once a target has been captured by a biomolecule, CB-Sherlock has been shown to be capable of achieving fingerprint identification of a single spore in less than one minute, without the use of reagents, labels, tags, or enzymatic 'amplification'.

CB-Sherlock is based on hyperspectral imaging microRaman. MicroRaman utilizes microscope optics to obtain Raman spectra from discrete areas (as small as 1um2), or pixels, of a surface. In imaging microRaman, light intensity is recorded as a function of both wavelength (the spectral domain) and location (the image domain). In the spectral domain, the data set includes a fully resolved spectrum at each individual pixel; while in the image domain, the data set includes a full image of all the pixels in the surface at each individual wavelength. Hyperspectral imaging is analysis in both the image and spectral domains. Recently commercialized hyperspectral imaging microRaman systems offer solid-state collection of a full spectrum at each pixel, with all pixels analyzed simultaneously, in seconds, even when spectra are collected from up to a million pixels.

To improve sensitivity and response times even further, CB-Sherlock exploits Surface-Enhanced Raman Scattering (SERS). The biomolecule pixels are immobilized on a SERS-active metal-coated chip. The SERS phenomenon can enhance the signals from the pixels by orders of magnitude over the normal Raman signal.

When the CB-Sherlock technology is fully developed, Biopraxis will work to couple an imaging microRaman with a tiny metal-coated biochip bearing a high-density array of biomolecule pixels. The detection process will consist of four steps: (1) The agent is captured by one or more of the biomolecule pixels; (2) Laser irradiation generates SERS spectra from the biomolecule-agent complexes at all of the pixels; (3) The spectra are collected from the pixels, individually and simultaneously, by imaging microRaman; and (4) the spectra are processed and analyzed.

Several types of information will be obtained. First, in CB-Sherlock as in conventional biochips, binding will be detected at pixels in the image domain, providing information about the types of biomolecules which recognize sample constituents. CB-Sherlock will go beyond conventional chips, however, by analyzing the chemical composition at each pixel simultaneously in the spectral domain to identify the targets captured by any given pixel on the basis of their spectral fingerprints. In addition, the different biomolecule pixels to which any given target binds will be determined by comparing the fingerprints produced at each pixel. Hence, CB-Sherlock can generate a binding profile in the image domain based on the biomolecule affinities for each of the targets, as well as generate fingerprints in the spectral domain for all of the targets that bind to any given biomolecule.

One noted difference that Biopraxis representatives pointed out is that, whereas conventional biosensor transducers are limited in the types of biomolecules that can be used, CB-Sherlock's microRaman can be used with any biological. This includes antibodies and enzymes, lectins and oligosaccharides, siderophores and hemes, peptides and receptors, lipids and aptamers, making it possible to detect agents from cyanide to anthrax with one instrument.

Biopraxis representatives stated that studies have shown that CB-Sherlock can detect nerve agents, toxins ranging from low-molecular-weight nonpeptides to high-molecular-weight proteins, and explosives with a high level of specificity. The SERS spectrum of a biomolecule is sensitive to perturbations caused by binding at a biomolecule's active site. Each different target - no matter how small - that binds to any given biomolecule will yield a different fingerprint, unique to that target. Because the SERS spectrum is based on the chemistry of the biomolecule-target complex, it cannot be produced by other sample constituents. Biopraxis representatives note that this means that the CB-Sherlock can therefore not just detect, but identify any toxin or CW agent even when cross-reactive biomolecules are used, and mixtures of cross-reactive toxins are present.

Similarly, the Raman spectra of microorganisms are fingerprint-like patterns that are highly reproducible and unique to different strains. CB-Sherlock studies have shown that SERS fingerprints can differentiate among the spores of closely-related Bacillus species, and even among the spores from different strains of anthrax. CB-Sherlock was shown to be capable of identifying the emerging bacterial pathogen Listeria monocytogenes at the subspecies level.

CB-Sherlock can be used to analyze complex samples with little or no sample preparation. The technology couples solid-phase extraction (e.g., immunoaffinity chromatography) with fingerprint identification by Raman analysis, done in situ on the solid phase. Company spokepersons noted that this means CB-Sherlock target identification differs from conventional Raman identification of unknowns in five key ways:

(1) In CB-Sherlock, the biomolecule is used to purify and concentrate the target. Chemicals and pathogens captured selectively by the different biomolecules at the different pixels are separated from the sample, and separated from each other, spatially, on the biomolecule array. Biopraxis calls the biomolecules in CB-Sherlock 'bioconcentrators (biocons),' in recognition that the many different types of biomolecules that can be used all function as a means to concentrate the targets of interest with high selectivity.

(2) Because SERS spectra of biomolecules are sensitive to perturbations caused by target binding, each different target that binds will yield a unique fingerprint. Some of the fingerprint is contributed by the target; some is due to changes in the chemistry of the biomolecule itself, caused by the binding event. In CB-Sherlock, therefore, the spectrum is collected from the biocon-unknown complex, and compared against a reference library containing spectra of biocon-known complexes, rather than the spectra from the knowns themselves.

(3) With conventional Raman, if several targets are to be monitored in a given sample, a single algorithm must analyze for all of them, in that mixed sample. In CB-Sherlock, however, the biocons will separate the targets spatially at the different pixels; and the imaging microRaman will collect the spectra from the biocon pixels individually. Hence, in CB-Sherlock, the algorithm used to identify one target can be quite different from that used to identify another - even when both of the targets were originally in the same sample and are captured on the same chip - and the algorithm used for any given pixel can be optimized for the biocon at that pixel.

(4) In conventional Raman, the identification algorithm compares the spectrum of the unknown against the spectra of all the knowns in the reference library. In CB-Sherlock, on the other hand, the library search associated with any given biocon will involve comparing the fingerprint of the unknown with just the fingerprints from the handful of compounds or microorganisms capable of binding with that one biocon. Hence, in CB-Sherlock, the algorithm for identifying any given unknown will be far less complex than the algorithm needed for identifying that same unknown by conventional Raman - yet far more reliable.

(5) In CB-Sherlock, information is collected in the image as well as in the spectral domain. Pattern recognition algorithms can be used at the same time spectral identification algorithms are used, to exploit information on cross-reactivities, specificities, and relative affinities, as well as spectral fingerprints, in identifying CB warfare agents.

The combination of biomolecule complexing, SERS, and microRaman makes it possible to achieve a high level of sensitivity and specificity, without any reagents, labels, or enzymatic amplification. Biopraxis representatives indicated that because the analysis is performed directly on the whole pathogen, rather than on its nucleic acids, none of the analysis cycle is wasted on sample preparation (e.g., on cell lysing, digestion, amplification, etc.) The SERS phenomenon is capable of enhancing the normal Raman signal without any of the disadvantages associated with resonance Raman or deep-UV Raman techniques.

Because the spectra are collected from all of the pixels simultaneously, the response time will be very short. Studies have shown that a unique SERS fingerprint, on which specific identification can be made, can be generated in less than one minute from a single anthrax spore. By using advanced identification algorithms, the spectra can be analyzed in a second or two.

Company representatives stated that the technology is flexible, and has the potential to be used in configurations ranging from a dipstick assay with fully automatic readout, to a fully automated, self-calibrating, real time monitoring system. Once the initial system has been fielded, CB-Sherlock can be upgraded for an almost limitless number of targets, by developing new biochips and algorithms. The microRaman readout system can be upgraded simply by reprogramming - i.e., without making any changes to the hardware. The CCD detector can be reprogrammed to collect spectra from pixels in different configurations; and the spectral reference library upgraded and the identification algorithms modified to match the additional targets. The software can be designed to accommodate the user entering new 'unknown' spectra and binding profiles into the reference library, in much the same way commercial spectrometer software can accommodate new spectra. As new agents are found that bind to CB-Sherlock's biocons, they can quickly and easily be included in future analyses.

The initial stages of CB-Sherlock development were funded through a Small Business Technology Transfer Program (STTR) awarded by the Army Research Office on nerve agent and toxin detection and a Small Business Innovative Research (SBIR) awarded by DARPA on explosives detection. Both Phase II contracts have just been completed. Under the STTR, CB-Sherlock was taken from a concept to a tiny

(~10mm2) biochip bearing as many as 8-10 biocon pixels that could be incubated for a few minutes in a 20µL sample, coupled with identification algorithms to automatically analyze spectra collected in 20sec. In a breadboard demonstration on more than 50 diverse environmental and food extract samples, the algorithms correctly identified cross-reactive aflatoxins, even when mixtures of the aflatoxins were spiked into the samples. Similarly, by the end of the SBIR Phase II, identification algorithms correctly detected and individually identified TNT, 2,4-DNT, and 1,3-dinitrobenzene mixed with soil heavily contaminated with nitrobenzene and/or 2,4-D.

The Department of Commerce recently awarded a Phase I SBIR on the detection of toxins in seafood, clinical samples, and seawater. To date, the technology has been shown to be capable of detecting and individually identifying histamine (scombroid poisoning, the most common cause of illness associated with seafood) and two other low-molecular-weight amines associated with spoilage, i.e., cadaverine and trimethylamine.

The U.S. Army SBCCOM recently awarded Biopraxis a SBIR Phase I on the detection of pathogens in foodstuffs. Initial studies showed that SERS fingerprints could be used as the sole basis for differentiating among cross-reactive species of Listeria; differentiating among different strains of a single species; distinguishing between cells of a single strain grown under conditions that induce or suppress the expression of virulence factors; and differentiate between viable and heat-killed cells. In addition, biomolecules associated with virulence (e.g., lectins and extracellular matrix molecules) were shown to be useful in developing binding profiles to help differentiate among strains; and in capturing pathogenic organisms selectively.

A SBIR Phase I for NASA targeted the simultaneous detection of inorganic and organic contaminants in water. Biochips capable of detecting and specifically identifying cyanide, ammonia, sulfate, sulfite, isopropanol, acetate, urea derivatives, and carbon tetrachloride were developed. Biocons were also demonstrated for the detection and specific identification of heavy metal cations and anions. Tests showed that metal ions could be individually identified in mixtures; and that the intensity of the spectral fingerprint was dependent on the metal ion concentration.

Phase II was recently awarded. Under this follow-on effort, NASA asked Biopraxis to expand the applications of its technology to include the detection and identification of viable spores on space flight hardware for use on the Planetary Protection Program. Under an IR&D project, Biopraxis had shown that the spores of four Bacillus stains (i.e., B. anthracis, B. brevis, B. cereus, and B. subtilis) could be individually identified on the basis of SERS fingerprints collected for 60 seconds. Three different strains of anthrax could also be differentiated from each other.

The programs have demonstrated the ability to use a wide range of different biomolecules in CB Sherlock.

Most recently, Biopraxis has received awards under DARPA's new Sensor Integration and Modeling of Biological Agent Detection (SIMBAD) and BioFlip initiatives. The Company has also just won a program for the Water Environment Research Foundation to develop a detector for Cryptosporidium oocysts and Legionella in water treatment and delivery systems.

Biopraxis spokespersons have point out that the CB-Sherlock technology can be coupled with more sophisticated biochip designs, using much smaller biocon pixels and analyzing much smaller samples. Under an IR&D, the Company has already shown that the biochip can be integrated with microfluidics devices for flow-through analyses and for studying biomolecular recognition processes in real time. Company representatives also noted that a number of approaches are available for improving CB-Sherlock's sensitivity and response even further.

The CB-Sherlock technology is interdisciplinary. Rather than attempting to bring all aspects of its development in-house, Biopraxis has teamed with strategic partners for capabilities in developing the various subsystems. Supporting technologies that their partners are developing include solid-state imaging microRaman, identification algorithm software, microfluidics, and computational fluid dynamics.

### *Bristol Industrial Research Associates & Limited*
### *Portishead, United Kingdom*

#### *Company Overview*

Bristol Industrial Research Associates & Limited (BIRAL), located in Portishead, United Kingdom, specializes in the supply and manufacture of scientific and industrial measuring instruments for a variety of applications in both high technology and mainstream industries. Founded in 1975, the company's business plan is to expand by seeking products that complement existing lines. There are now six key product groups – environmental monitoring, filter testing, fluid mechanics, meteorology, aerosol science, and industrial measurements.

BIRAL's Engineering Division manufactures BIRAL's own range of instruments, which currently includes visibility meters and aerosol shape analyzers. It also designs and builds customized measuring systems and test rigs, such as scanning filter testers.

#### *Technology Development*

BIRAL offers various particle size and shape analyzers, aerosol generators, and aerosol sampling systems. Company representatives claim that these instruments permit the measurement of particle size down to the nucleation size, thereby permitting the study of aerosol nucleation and growth. BIRAL addresses the measurement of sub micron particle size with a range of instruments which rely either on the electrical or diffusional mobility. Their products are outlined in the following paragraphs.

BIRAL offers Cascade Impactors, which are sampling devices in which the aerosol is divided into separate fractions based on the aerodynamic diameter of the particles and the amount of material in each of the size fractions is determined usually by gravimetric analysis. They have a range of models with cut sizes from as low as 0.056 microns and up to 32 microns. Cascade Impactors are best for use in mass distribution but they provide relatively low resolution information and are labour intensive and time consuming.

In their line of optical counter sizers, BIRAL supplies both conventional light scattering instruments and time of flight laser spectrometers. Company representatives noted that both types of instruments measure a number dependent on size distribution from which a mass distribution can be inferred by making certain assumptions. High resolution size distribution measurements over a size range typically from 0.5 to 30 microns can be accomplished in under a minute for data processing and presentation of the results.

BIRAL also sells non-invasive optical instruments, based on a range of techniques. These include phase Doppler anemometry, laser diffraction and scattered light intensity. Laser diffraction techniques rely on the diffraction pattern produced by light scattered from a cloud of particles and thus measure a size distribution but cannot give the size of individual particles. Laser diffraction instruments have been in use for many years for laboratory based measurements but BIRAL supplies systems engineered specifically for continuous on-line industrial process monitoring. The intensity technique that provides simultaneous velocity and size distribution information even from irregular and opaque particles. The technique has been engineered for the industrial environment and is suitable for the measurement of particle size and velocity distributions in such hostile environments as coal burning furnaces.

Company representatives stated that for particles smaller than one micron, inertial methods are not very effective and the scattered light techniques run into problems once the particles are smaller than the wavelength of light. BIRAL offers submicron sizing systems based on both electrical mobility and diffusional mobility. The Scanning Mobility Particle Sizer combines classification by electrical mobility with particle counting by a condensation particle counter to produce rapid high resolution size distribution measurements over the range from 0.005 to one micron. Typical measurement time is of the order of one minute and the sizing resolution is up to 64 channels per decade.

BIRAL offers a range of condensation particle counters which can be used as either components of particle sizing systems or as stand alone counters for submicron aerosols. The condensation particle counter overcomes the problem of detecting sufficient scattered light

from sub micron particles by using the particles as nuclei for the growth of liquid droplets and then counting the resulting liquid droplets. This technique provides a means of measuring the concentration of particles as small as 0.005 micron.

The different types of aerosol generation equipment available from BIRAL include:

- Vibrating Orifice Aerosol
- The Electrostatic Classifier
- Polydisperse Aerosol Generator.

BIRAL points of contact noted that characterizing particles by their shape is one possible method of identifying particles of a specific type against a background of other material. To date, the measurement of particle shape has usually involved collecting the particles on a filter and then analyzing them by optical or electron microscopy, sometimes with the aid of sophisticated image analysis systems. They consider such methods to be tedious and time consuming and produce only "after the event information."

The BIRAL particle shape measurement instruments' line uses the scattered light intensity profile to assess the size and shape of individual aerosol particles at rates of up to 10,000 particles per second. Their graphics software provides the user with a real time display of the particle size and shape distribution of the sampled aerosol from which changes in the aerosol characteristics can be observed.

***Bruker Daltonics, Inc.***
***Billerica, Massachusetts***

***Company Overview***

Bruker Daltonics Inc., located in Billerica, Massachusetts, is a Bruker affiliated company. The was founded in 1960 in Karlsruhe, Germany. In 1980, Bruker entered the field of MS by developing its first Fourier Transform mass spectrometer and by acquiring Franzen-Analytik GmbH which had developed a mobile mass spectrometer for emergency response and substance detection. With this beginning, Bruker founded a division that was to eventually become Bruker Daltonics Inc. - a global company focused on development, production and marketing of precision instruments, which are mainly based on MS technologies. Bruker Daltonics supplies instruments for biochemical, pharmaceutical and chemical applications. Today, Bruker Daltonics consists of 4 major centers including manufacturing and research and development: Bruker Daltonics, Inc., in Billerica, Massachusetts; Bruker Daltonik GmbH, located in Bremen, Germany; Bruker Saxonia Anlytik GmbH, located in Leipzig, Germany; and Bruker Daltonics GmbH in Zurich, Switzerland in addition to addition to sales and service subsidiaries in Canada, the United Kingdom, France, Scandinavia, Japan, and China. Bruker Daltonics Inc. was incorporated in Massachusetts in February, 1991, as Bruker Federal Systems Corporation. In February, 2000, they reincorporated in Delaware as Bruker Daltonics, Inc.

The company is considered a major developer and provider of life science tools based on MS. They are also a major supplier of MS-based systems for substance detection and pathogen identification in security and defense applications. Bruker has been involved in CB detection system work since the 1980s. Company representatives emphasized that they develop their CB detection systems from "the ground up", and do not tailor existing analytical instrumentation used in other areas to "force fit" the requirement. They feel they bridge the gap between developers and system integrators and design their systems to be modular.

Product revenue was $60.6M in 1999, an increase of $20.4M, or 51.0%, compared to $40.2M in 1998. This was partially due to an increase in demand for its life science products by industrial, academic and government customers. Currently, Bruker employs over 400 full-time employees, with approximately 80 employees in the United States and more than 320 employees located primarily in Europe. Over 100 of these employees hold PhDs in biology, chemistry or physics.

Instruments that the company provides include time-of-flight, ion trap and Fourier Transform Mass Spectrometry (FTMS) mass analyzers, in combination with MALDI and ESI ion sources. The Bruker Daltonics business also provides a line of mobile analyzers for environmental and chemical hazard monitoring, based on quadruple MS and other technologies. Their product lines integrate mass spectrometers with automated sample preparation and measurement and, where appropriate, bioinformatics software for use in:

- genetic variation analysis, including such evolving areas as pharmacogenomics and personalized medicine;
- proteomics;
- metabolomics;
- drug discovery based on high-throughput screening and combinatorial chemistry; and
- drug development.

Bruker Daltonics' customers also use their products in molecular biology and other basic medical research.

Bruker Daltonics manufactures the U.S. and NATO CBMS. The CBMS features short start-up times (20 min from –19°C), battlefield interference rejection (MS technology), BW agent detection and classification in 3.5 minutes, fully automated operation, and "button" user-interface via touch screen computer. The company has delivered over 50 units to the U.S. Army.

Other products the company provides in the CB detection systems market include the following:

- Reconnaissance/Anti-terrorism
  - -Fourier Transform Infrared (FTIR) Stand-off CW Detectors
- Personal Protection
  - -Handheld CW and Nuclear Detectors
- Treaty Verification/Demilitarization/Fixed-Site Monitoring

-Transportable and Remote Detectors
-Detector Networks.

Bruker Daltonics has a global life science customer base that presently includes over 400 customers. Their customer base consists of pharmaceutical, biotechnology, agricultural biotechnology, molecular diagnostics and fine chemical companies, as well as commercial laboratories, university laboratories, medical schools and other not-for-profit research institutes and government laboratories. They sell their substance detection and pathogen identification products and services to defense departments and law enforcement and emergency response professionals.

During 1998 and 1999, the ECBC accounted for 12% and 13%, respectively, of Bruker Daltonics' net revenue. Bruker Daltonics' production contract with the ECBC ended on March 31, 2000.

Bruker Daltonics markets its life science systems both through their direct sales force and through strategic distribution arrangements with Agilent Technologies, PerkinElmer, Sequenom, MWG-Biotech and others.

In June, 1999, Bruker Daltonics acquired substantially all of the assets of Viking Instruments Corporation, a developer and manufacturer of transportable gas chromatograph mass spectrometers. Customers use these instruments for laboratory and field analysis of soil, air and water for the identification and quantification of a wide variety of organic compounds and pollutants.

In 2000, Bruker Daltonics acquired ProteiGene, a biomarker R&D company specializing in the application of mass spectrometry and bioinformatics for medical and microbiology cell and tissue analysis.

Bruker Daltonics manufactures and tests the majority of their products in their three principal ISO 9001 registered manufacturing facilities located in the United States and Germany. Bruker Daltonics representatives stated that they have considerable manufacturing flexibility at their various facilities, and each facility can manufacture multiple products at the same time. With this flexibility, they doubled the capacity for their Time-Of-Flight system within one month. They stated that the facilities maintain in-house key manufacturing know-how, technologies and resources. Bruker Daltonics representatives also noted that they maintain multiple suppliers for key components that are not manufactured in-house.

### *Technology Development*

Bruker Daltonics maintains technical centers in Europe, North America and Japan. They allocate a significant amount of capital and resources (approximately 25%) to R&D and are party to various collaborations and strategic alliances. R&D expenses were $15.1M in 1999, an increase of $2.1M, or 16.0%, compared to $13.0M in 1998. The increase in 1999 spending was principally due to new products introduced in March, 2000. They expect to increase spending on R&D in order to continue to develop new products and applications.

Bruker Daltonics' MS-based systems often combine automated front-end sample preparation robots, advanced MS instrumentation, reagent kits and other consumables and bioinformatics software. Their systems are designed for use in such markets as genomics and proteomics, metabolic and biomarker profiling, drug discovery and development, molecular assays and diagnostics, molecular and systems biology and basic medical research.

Their systems incorporate four core MS technology platforms, including MALDI; time-of-flight MS; electrospray ionization, or ESI time-of-flight MS; FTMS; and ion trap MS. In addition to MS technology, for substance detection and pathogen identification, the company also has several other technology platforms including, ion mobility spectrometry, or IMS; quadrupole based GC, or GC-MS; fourier transform infrared detector, or FTIR; solid state radiation detection; and neutron activation detection, or NIGAS.

All of their products have potential dual use. Many of the systems, system components and methodologies produced by Bruker Daltonics are patented. Their current client mix for these systems are pharmaceutical companies, universities, SBCCOM, USMC, and the USAF. Approximately 40 percent of their current business is dedicated to government contracts.

Bruker representatives indicated that they design their products to address the evolving needs of the life science industry. Public and private efforts to sequence the entire human genome have led to advances that are fueling further investment in the discovery and identification of single nucleotide polymorphisms, and other forms of genetic variation. They noted that these developments, combined with other advances in combinatorial chemistry and basic medical research, are spurring growth in the following rapidly developing and emerging areas:

- PHARMACOGENOMICS, which uses genetic and genomic information to predict the response of individual patients and patient populations to drugs;

- PERSONALIZED MEDICINE, which seeks to apply inexpensive, rapid molecular diagnostic tests, or assays, to profile a patient's genetic composition and enable the prescription of individualized drug therapy;

- PROTEOMICS, which involves the large-scale separation, identification and characterization of proteins in order to understand how proteins are created based on the information contained in genes;

- NEW METHODS OF DRUG DISCOVERY, which are based on the high-throughput screening of large numbers of small organic compounds synthesized through combinatorial chemistry against large numbers of targets identified through genomics and proteomics;

- BIOMARKER DETECTION, OR BIO-BARCODING, which develops rapid and sensitive assays for a broad range of cell and tissue types for applications including infectious disease detection, human tissue assessment, agricultural phenotype differentiation and pathogen identification, even when the molecular mechanisms are not understood or the genomic sequence is not available; and

- METABOLIC PROFILING, OR METABOLOMICS, which analyzes the levels of metabolites present in a cell or in biological fluids to draw correlations between disease states, genetic modifications and variations in metabolite levels.

In addition, increased levels of funding for basic medical research have fueled demand by universities, medical schools and government agencies for sophisticated bioanalytical systems, such as mass spectrometers. Funding has also increased for substance detection and pathogen identification systems for security and defense applications.

Bruker Daltonics representatives indicated that many of the bioanalytical tools available today, other than mass spectrometry systems, have limitations when used for applications, including the detection of genetic variation, pharmacogenomics, proteomics, drug discovery and biomarker detection. These limitations include lack of throughput to accommodate the volume of analysis required, lack of automation, time-consuming sample preparation and insufficient accuracy of the resulting data. For example, the two leading methods traditionally used for DNA sequencing and expression profiling are electrophoresis and hybridization. The error rate of these techniques can increase the cost, complexity and time involved in completing more demanding analyses.

Bruker pointed out that they believe traditional protein science tools including Edman sequencing and two-dimensional gel separations are time consuming, relatively inaccurate and labor intensive. Additionally, many alternative life sciences tools can only be utilized by expert scientists. For other emerging applications, including metabolic profiling and rapid biomarker detection, Bruker Daltonics believes there presently are no automated, sensitive and accurate alternative tools available other than MS-based systems.

Bruker Daltonics is looking to solutions that address the limitations inherent in these alternative tools. Their product lines integrate mass spectrometers with automated sample preparation and measurement, and, where appropriate, bioinformatics software to address the bioanalytical and bioinformatics needs of the life sciences industry across a broad range of applications.

Bruker Daltonics is collaborating with Boston University on a hand held surface enhanced RAMAN spectrometer which could be applicable to BW agent detection. This research proposal had originally been submitted to the U.S. Army ERDEC in response to a solicitation for a chemical agent detection water kit. DoD will need 11,200 of these systems. Bruker

representatives stated that all of their system's components are COTS; that only the detection algorithms are proprietary.

Bruker Daltonics also has an agreement in place with the USMC for their Remote Air Pollution Infrared Detector (RAPID) stand-off detection system to be employed in the USMC's light reconnaissance system. The RAPID is a broadband infrared detection system for real-time remote sensing of hazardous atmospheric compounds.

Bruker Daltonics recently completed a five-year Advanced Technology Program grant from the National Institute of Standards and Technology for the development of a Mass Tag DNA Diagnostic Mass Spectrometer. They also have several ongoing, multi-year research grants from the German Federal Government.

***Cepheid***
***Sunnyvale, California***

### *Company Overview*

Cepheid, located in Sunnyvale, California, was founded in early 1996. Cepheid is a publicly-traded company which develops, manufactures and markets microfluidic systems that integrate, automate and accelerate biological testing. Their systems are miniaturized instruments that analyze complex biological samples in a disposable cartridge by combining molecular biology with state of the art fluidic technology that processes small as well as large quantities of liquid, appropriate for real-world applications. Some applications incorporate components fabricated with computer chip technology. These systems rapidly perform all of the steps required to analyze complex biological samples: sample preparation, amplification and detection.

Cepheid's goals are to reduce the size and increase the speed of key diagnostic instrument components and subsystems by combining micromachining technology with advances in instrument technology; then, to design and produce integrated bioanalytical test systems, with market applications ranging from human infectious disease and cancer diagnostics, food quality testing, environmental testing, and BW defense to R&D activities in molecular biology.

The company currently has over 100 employees.

Cepheid is initially focused on the detection and analysis of nucleic acids, such as DNA, in such samples as blood, swabs, urine, cell cultures, food and industrial water. The three key processing steps in nucleic acid testing are:

- Sample Preparation -- procedures that must be performed to isolate the target cells and to separate and purify their nucleic acids;

- Amplification -- a chemical process to make large quantities of DNA; and

- Detection -- the method of determining the presence or absence of the target DNA, typically through the use of fluorescent dyes.

Cepheid's systems perform a broad range of functions that include automated purification of DNA, screening for disease-causing agents, rapid detection of food and water contaminants, and genetic profiling. Their systems are designed for a wide variety of laboratory and field settings. Cepheid microDiagnostics™ systems cover a broad range of biochemical processing, from sample collection, to nucleic acid extraction and concentration, to nucleic acid amplification, to detection. The company's goal is to establish itself as a provider of systems that will allow practitioners in the life sciences research, clinical diagnostics, industrial testing and pharmacogenomics markets to make use of the vast new libraries of nucleic acid sequences now being generated by genomics researchers.

Key elements of Cepheid's strategy are:

- Apply core technologies broadly – The company intends to integrate their proprietary I-CORE and automated sample preparation technologies to provide rapid biological analysis platforms with applicability across a number of markets;

- Introduce products in stages – Cepheid intends to establish an initial market position in the life sciences research market by providing a fast, flexible thermal cycler, their Smart Cycler. Company representatives stated that their next product, the GeneXpert system, will fully integrate and automate sample preparation with amplification and detection;

- Focus initially on nucleic acid analysis – The company is initially focusing on the development and application of their platform technologies to the field of rapid nucleic acid analysis. They will adapt their sample preparation and amplification technologies to increase the number of samples that can be processed, referred to as throughput, as well as lower costs and provide greater sensitivity.

Some of Cepheid's technologies are at development stage. Cepheid began commercial sales of their first product, a thermal cycler with real-time optical detection called the Smart Cycler, during May, 2000. The Smart Cycler is a DNA amplification and detection system initially directed to the life sciences research market.

Cepheid's GeneXpert system, currently in development, is designed to integrate automated

sample preparation with their Smart Cycler amplification and detection technology in a disposable cartridge format. This integrated system will allow the fast analysis of biological samples. A prototype of the system was delivered to Lawrence Livermore National Laboratory (LLNL) in November, 2000. The company is collaborating with strategic partners to co-develop assays as well.

Cepheid completed their initial public offering on June 21, 2000. Net proceeds from the sale of the shares of common stock were approximately $31M. Their historical net tangible book value as of September 30, 2000 was approximately $44.4M. Since inception, Cepheid has incurred significant losses and, as of September 30, 2000, had an accumulated deficit of $24.2M. They anticipate incurring additional losses, which may increase, through at least 2002 as they increase their commercialization activity. Their losses have resulted from R&D, manufacturing scale-up and selling, general and administrative costs associated with their operations. They also expect to incur increasing R&D and manufacturing scale-up costs. Cepheid's revenue, prior to the launch of the Smart Cycler, had been derived from grants and government-sponsored research, and R&D contracts with commercial partners. Since that time, Cepheid's revenue has consisted primarily of product sales.

On November 17, 1998, Cepheid and Innogenetics N.V., a Belgian biotechnology company, executed a development and supply agreement focused on the application of Cepheid technologies into systems and consumables that optimize the performance and ease of use of Innogenetics test methods for infectious disease and genetic disease diagnosis. Innogenetics has exclusive rights to distribute the resulting products on a worldwide basis. In a related transaction, Innogenetics made an equity investment in Cepheid, which at the time, represented five percent of the shares of Cepheid.

In May, 2000, Cepheid and Infectio Diagnostic (IDI) Inc., of Sainte-Foy, Canada, formed a joint venture, ARIDIA Corp., to commercialize products in the field of rapid human infectious disease testing. The products to be developed by the joint venture will utilize Cepheid's proprietary, integrated systems for rapid, automated sample preparation, analysis, and detection, and IDI's portfolio of proprietary molecular diagnostic reagents and methods.

ARIDIA (Automated Rapid Identification and Detection of Infectious Agents), a Halifax, Nova Scotia, based corporation that is equally owned by Cepheid and IDI, is developing a line of tests and test systems to enable the time critical identification and detection of bacterial and fungal infections, including Group B strep, antibiotic resistant bacteria, meningitis, Candida, and septicemia. The products will be sold initially to hospitals and private laboratories.

### *Technology Developmnet*

Under a two-year, $2.4M contract with the ERDEC, Aberdeen Proving Ground, Maryland, Cepheid designed and delivered a micro-fluidic, fully-automated DNA analysis system for the on-site detection of bio-warfare agents in less than 30minutes.

The system, Micro-fluidic Integrated DNA Analysis System (MIDAS), was designed for use in a variety of laboratory and mobile detection scenarios to automatically detect and identify bio-warfare agents. The instrument uses advanced micro-fluidic circuits to automatically process fluid samples suspected to contain bio-warfare agents such as anthrax. Cepheid's proprietary micro-fluidic circuits automatically perform the processing steps required to filter the sample, rupture any biological organisms, accurately mix the sample with the required reagents, purify the DNA, and transfer the purified sample into the Cepheid I-CORE (Intelligent, Cooling/Heating Optical Reaction) module (patent pending).

Once inside the I-CORE, the sample undergoes PCR. PCR amplifies, or creates new copies of target DNA or RNA sequences through a process of heating (denaturing) and cooling (annealing) the original bio-warfare DNA molecule in the presence of specific DNA primers, enzymes, and reagents. Each thermal cycle produces a near doubling of the target DNA (or RNA) segment, leading to an exponential amplification of the target DNA for subsequent identification. Using I-CORE, heating and cooling can be done very rapidly - in minutes.

The MIDAS employs Cepheid's microDiagnostic™ technologies, which include designs for fast chemical reactors patented by the

LLNL and licensed to Cepheid for nucleic acid amplification and detection. The system also incorporates Cepheid's proprietary thermal cycling, optical detection, and micro-fluidics technologies.

In May, 2000, Cepheid received an additional $1.8M from the ECBC to develop a man portable, expanded capability MIDAS with developed assays. This effort will miniaturize the current fully automated MIDAS prototype to a man-portable weight and size (i.e. backpack), expand its capabilities from one to eight independently programmable assays, and incorporate a sonication based sample preparation system. The unit will be fully automated and operate continuously over a twelve-hour unattended mission or on-request.

In 1998, Cepheid, along with two consortial partners, received a $5M, three-year grant from DARPA to design and manufacture advanced, miniaturized diagnostic systems for rapid detection and identification of biological pathogens. The system being developed by Cepheid and its collaborators — the USAMRIID (Fort Detrick, Maryland) and Vysis, Inc. — will employ microfluidic and microengineering technologies, molecular-based screening and diagnostic algorithms, and powerful chemistries to enable sample handling, target purification, amplification, and detection of multiple pathogens in a portable instrument.

Cepheid, the leader in this consortial effort, is tasked with developing active micromachined microstructures, fluid processing cartridges, and instrumentation for automated target pathogen and nucleic acid purification systems. Vysis is working with Cepheid to integrate their novel multiplexing detection technology into diagnostic fluidic cartridges. The USAMRIID, the lead DoD laboratory for medical biological defense, is responsible for all diagnostic testing for the U.S. Army and is defining specimen-processing and assay protocols and validating the assays and instrument systems that are created.

Cepheid was also awarded a $750K Phase II SBIR grant in 1998 to further its work on developing a portable, high-speed PCR thermal cycler. The instrument is intended to alert military personnel to the existence of pathogenic agents in the field. The grant is funded by the DoD and sponsored by USAMRIID.

Cepheid had received a total of $1.3M under SBIR grants to develop a portable, battery operated thermal cycler for diagnostic testing in the field. This system, the Smart Cycler XC integrates a separate computer controller with the analytical thermal cycling instrument to produce a multi-test, portable instrument packaged in a briefcase.

Cepheid has received an additional $550K under the SBIR grant, to deliver two prototype spore lysis and detection systems. These systems will integrate automated sample processing and rapid quantitative nucleic acid analysis into a disposable cartridge.

Cepheid representatives perceive possible commercial applications for the new instrument include point-of-care diagnosis of infectious and genetic diseases, on-site testing of food for contaminants such as *E. Coli* and salmonella, and field testing of air and water for sources of infection or pollution.

### *Computing Devices Canada*
### *Calgary, Alberta, Canada*

### *Company Overview*

Computing Devices Canada (CDC), with offices in Calgary, Alberta, and Nepean, Ontario, is an international supplier of land, airborne and maritime systems, software and hardware. Their work experience includes:

- Integrated digital voice and data distribution
- Acoustic signal processing
- High resolution tactical digital displays
- Flat panel displays
- $C^3$ systems design and integration
- Multi-sensor scan conversion
- Ballistics
- All-weather, multi-spectral surveillance for air, sea or land
- Integrating COTS technology
- Digital fire control systems
- Information technologies
- Software development
- Communications engineering
- Sensor integration.

CDC was formed in 1948. In 1997, General Dynamics Corporation acquired CDC for $C600M. Computing Devices Canada operates as a wholly-owned subsidiary of General Dynamics. They have facilities in Minnesota; Calgary, Alberta, and Ottawa, Ontario, Canada; Hastings, United Kingdom; and an office in Washington, DC. CDC has current annual sales of $C400M.

CDC has approximately 3300 employees, 430 of whom are located in Calgary and are developing chemical- and biological-agent detectors and mine detectors for the U.S. and Canadian military, as well as a network linking health specialists to remote areas.

CDC's customer breakout is 90% military, 10% commercial. Of military clients, 77% are U.S. Army, 18% are USAF, and 5% are USMC. Their USMC customers include the Direct Air Support Center and the Advanced Amphibious Assault Vehicle office. Of military contracts, about 70% are foreign, 30% Canadian. In addition, CDC has over 20 international customers.

In the biological detection system area, CDC intends to concentrate on providing florescence-based biological detectors. No longer considered systems integrators (as they were on CIBADS), they will sell a complete biological detector system as well as just the detector instruments. Improvements the company is working on for their fluorescence particle sizers include reductions in the size, weight and cost of this system. CDC envisions that potential customers for these systems include the pharmaceutical and medical diagnostic markets, first responders, military, U.S. Secret Service, Federal Bureau of Investigation, fire departments, airports, embassies, and hospitals.

CDC's involvement in biochemical agent detection and identification stems from its work in response to the Canadian Forces' program for a CIBADS. As the lead of an Integrated Product Team, Computing Devices worked with the DRES; the University of Alberta; the CF; Scientific Instrumentation Ltd (SIL), Saskatoon, Saskatchewan; Dycor, Edmonton, Alberta; and TSI, Minneapolis, Minnesota, to produce the CIBADS Advanced Development Model.

CDC, Calgary Operations, has been awarded a contract from DND for two 4WARN Mod 1 Biological Detection Systems. The contract will allow DND to use the 4WARN Mod 1 Biological Detection System during operational deployments, as well as to evaluate the system as part of its procurement strategy. The 4WARN System is a spin-off from the CIBADS II Advanced Development Model program being developed by Computing Devices and currently in its final project phase.

CDC's Bio Defence Systems was awarded a contract from the U.S. Defense Threat Reduction Agency (DTRA) for participation in a Smart Building Biological Sensor Demonstration in February, 2000. The demonstration involved integration of the 4WARN Urban into the DTRA Consequences Assessment Tool Set (CATS) and participation in trials at a location near Salt Lake City, Utah. Participants in the trials included DTRA, hazardous materials responders, FBI, and U.S. Secret Service representatives. This exercise is part of DTRA's preparation activities for the Salt Lake City Olympics. CDC has also been recently awarded a contract to supply a system to Egypt for evaluation and possible procurement.

### *Technology Development*

CIBADS has been tested both in trials and in actual operational deployment situations, including:

- At the U.S. JFT at Dugway, Utah, in October, 1997, where the system achieved a perfect score.
- Deployed on the HMCS Toronto in the Persian Gulf, featuring 1500 hours of operation (late 1997 -early 1998)
- Deployed at the Asia-Pacific Economic Cooperation (APEC) meetings in Vancouver in November, 1997.

CIBADS forms the basis of the 4WARN family of real-time biochemical agent detection and identification systems. CDC's 4WARN detection and identification systems are the product of the integration of COTS biological and/or chemical sensor technologies. CIBADS (and 4WARN) consist of the following components:

- FLAPS for real time bio-detection - DRES licensed the manufacture of FLAPS to TSI (a U.S. company )
- Dycor XMX liquid sample collection
- ATR for bio-identification (optional on 4WARN)
  (*Note: Dycor manufactured the ATR but the design (and software) is CDC's)
- ICAM chemical detector (optional on 4WARN)
- Vapor sampler
- Central processor with sophisticated software to control the system, provide the alarm algorithm for the FLAPS, and send the alarm message to a remote host
- GPS and meteorological instrumentation.

4WARN/CIBADS use FLAPS technology (Details of this technology are provided in Section 4). CDC researchers believe that FLAPS is the only detector that can discriminate between biological particles and other particles in air in near real-time. Currently, a FLAPS 2 costs $US167K from TSI (although CDC purchases them for slightly less). The cost of a complete CIBADS configured 4WARN is $US450K.

CDC's goal is to get away from heavy, very expensive military biological detection systems. They are looking to move away from just military users to commercial ones but cost must come down. The cost of 4WARN is too much for commercial applications. CDC is looking at alternate core technologies costing about one-fifth the cost of the present system.

To reduce the cost, weight, size, power and cooling requirements of FLAPS (the core 4WARN technology), CDC has partnered with Pacific Scientific Instruments (PSI), Grants Pass, Oregon, and has developed the Biological Agent Real Time Sensor (BARTS). BARTS can be used for detecting biological agents; monitoring the airborne spread of disease-causing organisms in hospitals, food processing plants, large buildings, or pharmaceutical facilities; and determining the mechanisms for spreading of airborne particles. The CDC-PSI partnership was one of five companies short-listed for North America's Best Technology Partnership, an awards program launched by the Canadian-American Business Council and Canadian Advanced Technology Alliance (CATA Alliance), Ottawa, Ontario. BARTS (without the collector) is about 4 inches x 12 inches x 10 inches in size, and should cost "a few tens of thousands" of dollars.

CDC has designed a commercial vehicle-mounted biological detection system, called the 4WARN Urban concept, based on their 4WARN technology. The 4WARN Urban biological detection system is designed for easy integration within standard sport utility vehicles with minimum vehicle modifications. The current configuration, designed for biological agent response, is expandable to include nuclear and chemical detection, as well as other NBC functions.

4WARN Urban is composed of a range of integrated sensors, sampling equipment, positioning and meteorological equipment, communications equipment, and processors for real-time detection, alarming and messaging functions. These components include:

- Fluorescence aerosol biodetector
- Particle concentrator
- Embedded computer
- GPS receiver
- Meteorological station
- Secure satellite communications
- Interior workstation with laptop computer
- Custom roof-mounted sports pack enclosing sampling equipment and antennae.

### *CyTerra Corporation*
### *Waltham, Massachusetts*

### *Company Overview*

CyTerra Corporation offers a unique capability to address complex chemical, biological and hazardous material detection issues. Formerly the Thermo Electron R&D Center Inc., CyTerra is now an independent company focused on leveraging R&D into viable commercial products. The company is made up of a unique multidisciplinary team of senior scientists and engineers with extensive expertise and experience in all areas of analytical instrumentation, environmental characterization and personnel/worker safety. CyTerra has developed many new instruments and technologies over the past 20 years that have helped propel Thermo Electron Corporation to its position as the largest analytical instrument company in the world.

A review of CyTerra's past experience, products, and R&D programs shows a broad experience and knowledge base unlike any other entity for conceptualization, design, implementation and manufacture. It's technical expertise includes contaminant sampling systems, high-speed GS systems, air sampling, nuclear detectors, pollution monitors, health physics, worker safety, X-ray analysis, detection of trace levels of hazardous metals, trace analysis of organics such as PCB's, explosives, and carcinogens, spectroscopy, laser physics, microwave technologies, ground penetration radar, RF communication, signal processing, data fusion, imbedded software, personnel monitors, and worker safety.

Many specific modular sensor systems have been produced for the military, government agencies, and commercial industries as a direct result of CyTerra's accomplishments. Commercially developed products have typically set a new standard of performance and excellence that have led to numerous awards for effectiveness and functionality, including The Presidential Design Award for Technical Excellence (presented at the White House by President Clinton in 1995). CyTerra's employees have been awarded nearly 100 U.S. patents. Another yardstick of CyTerra's world-class capability is that their commercial products dominate the international instrument market place.

CyTerra's mission is to continually search for opportunities to apply its expertise in emerging technologies to find solutions to real-world problems. They intend to further enhance their position in diagnostics and disease detection as a result of their recent advancements in CB sampling and analysis work. Additionally, CyTerra is working with Battelle to develop environmental agent detectors with similar technologies.

### *Technology Development*

CyTerra is currently developing a system to monitor ambient levels of CB agents for a fixed base application - not a battlefield application. Vapors and particles are passively collected and then analyzed with GC and pyrolysis. The approach is to detect and identify bioagents based upon a reduced sulfur signature from amino acids. The sample is analyzed using a micro oven that can heat samples within one second. The high-speed GC MS detection system is capable of ultra-fast measurements. Vapor collection is used for chemical detection; particle collection is used for biological detection. The system compares real-time readings to background signals gathered over a longer period of time to identify changes in the quantity of vapors or particles collected.

A system to accomplish this analysis is projected to measure one cubic foot, weigh approximately 20 pounds, requires less than 100 watts, and provide video and audio alarms and LAN connections.

In a related technology, CyTerra has developed a non-intrusive method to monitor personnel exposure to ambient air contaminants. The system could be tuned to identify sulfur, phosphorus, chlorine or nitrogen compounds as key markers. As a result of this work the company has determined that exhaled breath might also provide a diagnostic measure for disease based on molecular signature of infections or metabolic signatures of medical conditions.

CyTerra is also working with the USMC on a Chemical/Biological Individual Sampler (CBIS) that could sample ambient air for sub-clinical exposure levels of warfare agents. The system uses a passive sampler worn by an individual for an extended period of time, e.g., 24 hrs to 7 days. The dosimeter badge consists of a patchwork of

adsorbents and particle traps. The typical analysis time is projected to be ten seconds, allowing hundreds of CBIS to be analyzed per day. They anticipated that the sampler portion of the detection system would cost about $1, but that the total cost of the system is yet to be determined. The detection system is designed to allow for easy interface with a variety of detectors. Company representatives envision this system could be integrated with a GPS and cellular communications with programmable mapping software and a single device could be used per group of deployed troops. In this way troop movements could be mapped and tracked for development of the exposure history. The CB sampler could also be configured for civilian applications such as for use in workplace monitoring.

R&D presently being conducted at CyTerra will result in a fundamental technology base for use in future products for CB analysis.

### *Dycor*
### *Edmonton, Alberta, Canada*

#### *Company Overview*

Dycor is a privately held corporation originally established in 1981 to supply custom research equipment and technical support to universities, government labs and the military. Dycor's corporate office is in Edmonton, Alberta, and it also has offices in Edgewood, Maryland, and Colorado Springs, Colorado. Dycor currently employs 30, but a company spokesperson stated that they are planning to return to 40 staff towards the end of 2001. Dycor's current annual sales are $C5M.

A company spokesperson noted that Dycor wants to position itself as "purveyors of good, solid information" and to build on the relationships with Defence Engineering Research Agency (DERA), Dugway and DRES to "become a clearinghouse of information". He stated that it is not their goal to build large scale "green boxes" or compete with the "green box" builders. The company's scientists and technologists are focused on providing solutions to a wide range of problems associated with aerosols, fluidics, optics, telemetry, and electronic measurement and control. Dycor is committed to attracting world-class scientific and engineering personnel; investing in state of the art equipment; gathering market intelligence; and building multidisciplinary strategic alliances.

Dycor is trying to develop tools and expertise to allow independent testing of manufacturers' claims. The company's goal is to create a separate division with its own controls and financing that can be recognized as an unbiased test agency. Dycor also wants to provide training, doctrine, instruments and backbone systems to allow smaller countries and organizations to develop expertise and policies.

Our newest division will focus on physical as well as NBC vulnerability analysis for government agencies and owners of hardened facilities or sensitive installations.

Dycor's major clientele consists of government agencies and Fortune 500 firms. The company supplies different system components to the USAF, U.S. Army, DERA UK, and the French MOD. Dycor representatives noted that they also provide and maintain the backbone BW T&E referee system at Dugway. Other customers include customers include the R&D departments of all major Canadian universities and technical colleges; government research laboratories; the petrochemical and oilfield services industries; public utilities; and the aerospace industry. Dycor scientists are working with DARPA, as well as with military agencies in Germany, Sweden, France, Korea, and Japan.

Dycor believes that foreign countries see direct, immediate threats and therefore have money to invest in solutions.

Dycor does not seek patents on its products at this time. A company spokesperson described patents as "licenses to litigate" and said that Dycor simply does not have the financial backing to defend its designs in court. It relies on trade secrets and alliances with strategic partners rather than patents to protect its intellectual property.

#### *Technology Development*

Dycor partnered with GD/CDC and DRES on CIBADS. Dycor performed two critical tasks for CIBADS – FLAPS 2 and XMX. DRES contracted with Dycor and TSI to combine the TSI APS with a 355 nm laser for bio-fluorescence detection. In addition, Dycor developed the Bioaerosol Detection Software – the vital Intellectual property - for the FLAPS 2 used in the detection of biological weapons of war. The FLAPS 2 has been judged one of the top 100 most technologically significant new products of the year by the U.S. magazine *R&D*. Dycor, DRES and TSI shared this award. Dycor also provided a refined aerosol concentrator/liquid sample collector (the XMX) for CIBADS and 4WARN.

Dycor manufactures and sells the XMX, an actual product evolving out of the XMX program for the R&D of sample collection, concentration, refining, and distribution technology. This area is critical to the success of any BW detection and identification technology. Delivering a contaminated sample to any analytical instrument will in most cases render it incapable of making an accurate assessment of the threat. Dycor's XMX Virtual Impactor is an aerosol concentrator/liquid sample collector. As an aerosol concentrator, the XMX samples large volumes of air and concentrates the air stream

for output into a variety of instruments. The XMX operates as a three-stage concentrator and collects particles ranging in size from sub-microns to greater than ten microns, depending on the configuration. When coupled with the Liquid Impingement Module (LIM), the concentrator provides concentrated liquid samples that can then be used for batch or continuous flow analysis. In this configuration, the XMX directs a particle enriched flow stream into a sample collector vial containing an application-determined volume of liquid.

Operation of the XMX is straightforward and does not require users to possess a technical background. It can be pre-configured for maximum efficiency of particle collection based on the user's requirements. For more sophisticated research applications, additional options exist to help the scientist/researcher fine-tune the instrument and make adjustments as required. This includes the ability for the XMX to communicate with the attached instrument for the purposes of optimizing its operation or protecting itself from damaging conditions.

Dycor contracts out volume manufacture (current in house production capabilities "in the hundreds"). Dycor manufactures the "backbone" for biological detection systems – the threat detection system software combined with line-of-sight spread spectrum radio modems, met/GPS stations, and custom interfaces for CB detectors. Although they do not manufacture detectors at this time, they would integrate a suite of sensors provided by a customer or even deliver a complete system to a customer's specs. Dycor's products include sensors, components, engineering support and spread spectrum digital radio for 30, 100 or 1000 miles (radio modem).

Dycor is continuing to conduct R&D on the XMX to better enhance the system, including:

- Reducing the size, weight, and power consumption of the instrument
- Developing a continuous liquid flow particle impingers
- Reducing and/or eliminating the amount of liquid required to impact particles
- Adding of a HEPA filter
- Reducing decontamination requirements.
- Adding sample cleansing and distribution

Expertise obtained by Dycor in the creation and implementation of chemical weapons detection systems for U.S. and Canadian defence departments was applied to develop a commercial version of the CB/Net and HPAMS sensor communications network. Dycor has produced the CB/Net to provide for the detection and identification of hazardous materials. The company envisions that the CB/Net will also be useful in situations where there is a perceived threat of attacks by terrorists using chemical or biological weapons as well as a general purpose environmental monitoring system and training tool. This makes the system of value to the end user on a continuous basis ensuring the system and attending personnel are always in a ready state for more serious situations.

The CB/Net HPAMS is a universal platform equipped with a basic suite of sensors for meteorological, and GPS positioning data. Instrument interface and carrier modules have been developed for particle detectors, chemical sensors, and other user specified devices. Interfaces for propylene and SF6 tracer gas detectors are under development to allow for training and testing of the system, as well as for marker gases during site remediation. For more sophisticated applications, the system can be equipped with high resolution APS and FLAPS.

The CB/Net HPAMS is designed to be used as a perimeter monitoring system. Several units deployed around the perimeter of a site monitoring for potentially dangerous gases and or airborn particles and reporting to a central base station. Each remote transmits its data, along with GPS co-ordinates, to the host computer at the monitoring station or command center. Here the information is logged and monitored using our CB/Net software (Microsoft Windows™ platform). Users can set alarm points in the software and remote audible/visual alarms can be triggered automatically if desired.

Dycor has developed CB/Net, a Microsoft Windows™/ National Instrument LabView based software platform that communicates with a variety of instrumentation used in the CB agent detection arena. The software allows the user to simultaneously communicate with, display, store, control, and analyze, signals coming from instruments such as aerodynamic particle sizers, airborne and liquid flow cytometers, meteorological stations, GPS, IMS, air and liquid samplers.

The system allows the user to work with standard software and hardware platforms that they may already have in place including networks. Radio telemetry remote modules allow sensors to be placed in distant locations where hazards may exist.

Taking GPS data from the remote devices, the system can display the location of the remote devices overlaid on maps of the area supplied by GIS maps or similar systems. Icons representing each remote unit appear on the computer screen at their respective GPS coordinates. When a suspicious or threatening condition has been determined by alarming algorithms and set-points in the server computer the icon representing the remote sensor will either change color or flash as well as sounding a general alarm to the computer operator or any other system or battle group connected to the system through the network. The operator can select the icon and open it revealing the description of the remote sensor and the exact nature of its alarm. The operator can select and then view detailed data from the instrument as well as control remote systems. Information from the server computer can be relayed to other computer stations on the network or to remote locations via satellite or radio modems. Remote users can also take control of the system and send relevant data off via telephone modem, digital radio, or the Internet, to other computer systems or experts for analysis. Decisions can then be made by the remote users and control commands or advice can be sent to the battlefront as required.

Dycor intends to expand the markets for its COTS system to cover the protection of strategic industrial and commercial centers against terrorism as well as monitoring public and private buildings for environmental hazards. Continuing R&D focuses on hardening the system for industrial and pseudo military uses and increasing the computing power of the remote sensing systems, allowing autonomous operation and improved detection and identification capabilities.

New research efforts will focus on unique RF, MEMS, Microwave, and Hyperspectral imaging sensors for the detection and identification of CB threats.

### *FemtoScan Corporation*
### *Salt Lake City, Utah*

#### *Company Overview*

FemtoScan Corporation, founded in 1990 and located in Salt Lake City, Utah, is involved in environmental research (chemical detection and monitoring) and instrument development. This small company's main focus is on Automated Vapor Sampling-Transfer Line Gas Chromatography (AVS-TLGC) technology. The technology was developed by the founders while they were employed at the University of Utah, Center for Micro Analysis and Reaction Chemistry, and FemtoScan has negotiated an exclusive worldwide license from the university.

The company currently offers its AVS-TLGC technology in two forms:

- the Enviroprobe module, and
- the Environmental Vapor Monitor (EVM) II.

#### *Technology Development*

The Enviroprobe module is a small (6x6x4 inch), fast (1-30 second analysis time), automated sampling GC system that is meant to be a "front-end", add-on to a detector of the user's choice (MS, FTIR, GC, etc.). By coupling the Enviroprobe to a detector, the user will have the capability of repetitive, automated sampling directly from the environment. Using their patented sampling introduction method, the sample is directed immediately onto the capillary column.

The Enviroprobe was developed to address a number of analytical problems expeditiously by employing on-line or in-situ sampling. According to company representatives, in real-world applications, this is easier said than done. Real-world samples involve mixtures. In order to separate and quantitate mixtures of analytes and interferants, the analytical chemist requires either off-line sample preparation or powerful on-line analytical methods capable of separating the target constituents. The Enviroprobe is designed to apply the latter approach to gas phase analytes in a way that is consistent with the on-line, in-situ, real-time, on-site philosophy.

The Enviroprobe module is a fully-automated, pulsed, direct atmospheric vapor sampling unit (the AVS), integrated with transfer-line capillary gas chromatography (TLGC). Together, the combination of AVS-TLGC allows a vapor phase analyte to be directly coupled into a capillary column without the need for intervening valves or other adsorption site contaminants. Automated pre-set valves pneumatically move carrier gas through the system drawing the sample directly into the transfer line.

Enviroprobe units are used for a number of commercial MS, IMS and Fourier Transform Infrared detection systems. Capabilities include on-demand, repetitive, or computer controlled sampling at intervals from one second to one day. The Enviroprobe weighs less than three pounds (without external power supply), measures 6x5x5 inch and operates off a 12 or 24 volt external power supply.

The EVM II instrument incorporates the features of the above described Enviroprobe technology with IMS IMS to produce a hand-portable, hyphenated spectroscopic technique. The EVM II is a commercial GC/IMS instrument for process and field applications. The EVM II was developed as a SBIR contract for the ERDEC. It is based on the IMS technology for ultra sensitive detection of gas phase analytes with high speed AVS-TLGC sampling and separation capabilities. The EVM II is a near real-time vapor detector that uses intelligent AVS injections, rapid TLGC separations and high sensitivity IMS detection.

The EVM II is a detector system for portable applications. The lightweight package operates from a 24 volt battery pack or from an external power supply. At power-up, the instrument performs a self-diagnostic, resets the sampling and GC conditions to the previous values, comes up to temperature and is ready for operation within 15 minutes for most applications. Sampling operation is either on-demand or continuous, allowing different degrees of operator interaction based on the application. All sampling and GC parameters can be reset via the notebook computer data system. Designed for the constraints of field use, the EVM II allows for the use of multiple AVS-TLGC modules in the field with a rapid change capability for AVS-TLGC "front ends." Responses can be

observed via the on-board liquid crystal display or via a portable notebook computer data system.

Company representatives noted that the EVM II is immune to water vapor, allowing the EVM II to be a "full range" detector for volatile organic compounds, many of which are not detectable with conventional direct IMS. The low sampling duty cycle of the AVS system reduces water vapor loading to levels that allow the direct detection of alkanes and other organic species typically not detected by IMS systems.

Potential EVM II applications include:

- CB weapons detection
- pesticide analysis
- environmental monitoring
- process monitoring
- stack monitoring
- leak detection
- worker exposure determination
- quality assurance and quality control..

### *IatroQuest Corporation*
### *Ottawa, Ontario, Canada*

#### *Company Overview*

IatroQuest Corporation, a small, private Ottawa-based Canadian company, spun off from the National Research Council of Canada (NRC), has made advances in developing rapid sensing and diagnostic 'smart materials' enabling for systems used in the detection and identification of CB warfare agents. The company, founded in January, 1999, is focused on developing next generation bio-sensing and ultra high throughput diagnostic systems for military and civilian defence, medical, bio-pharmaceutical and environmental monitoring applications. IatroQuest's core platform technology, termed Bio-Alloy 'smart materials', is designed for integration into end-user biosensors or diagnostic devices. Product commercialization and distribution will be accomplished within strategic alliances with key sector players. IatroQuest currently employs ten people and is undergoing rapid growth. This core R&D team is supplemented by work conducted with contract research organizations.

Currently, IatroQuest has patents pending worldwide for its Bio-Alloy™ platform technology. This technology combines elements of biotechnology, advanced materials and photonics yielding unique biosensing properties. According to company representatives, Bio-Alloy™ materials allow sensitive, selective, real-time detection and identification of biological agents in a rugged, cost-effective format. The cost of devices, which will integrate the smart sensing materials, have not been determined but IatroQuest is working to meet a DARPA wish list of $US5K or less and 5-10 pounds in weight. This approach is being taken in order to increase the distribution of detection devices across a potential threat area. IatroQuest is currently conducting R&D on the Bio-Alloy™-based sensing modules. Production is expected to start in early 2002. Company representatives believe this technology can be customized to meet the sensing requirements for a variety of applications (e.g. medical diagnostics, environmental monitoring) in various device configurations. They indicated that the technology lends itself to be used in miniaturized, portable, biosensing devices that can be used in defense theaters for real-time CB warfare agent detection and identification. IatroQuest researchers came across their discovery while working on studies exploring the integration of biomolecular structures with semiconductor materials.

IatroQuest has received a $C500K Canadian Defence Industrial Program (DRDC DIR) grant for R&D of Bio-Alloy™ smart materials and is hopeful to receive a second such grant in 2001.

#### *Technology Development*

IatroQuest starts the manufacture of Bio-Alloy™ with semiconductor materials (e.g. silicon) which can take various forms including ‘planar microchip-like’ or microparticle form. The company currently uses 200-micron silicon particle format. They first use a proprietary chemical etching and surface chemistry process to produce a controlled microstructuring on the material suitable for attaching biomolecular receptors. Biological recognition elements, including antibody fragments ("hooks"), are then immobilized onto the microstructured material. Any recognition element available now or in future can be bound to the microstructured material. This microstructured material with bound biological recognition elements is collectively termed Bio-Alloy™. A low energy blue light (under 10mW CW laser or LED) is used to excite the Bio-Alloy™ material surface. The non-activated core microstructured silicon material normally emits light in the visible red-orange spectrum. If the Bio-Alloy™ material is exposed to a biological agent matching the recognition element, that agent binds yielding a change in photonic 'signature' in the green visible part of the spectrum. Alteration of the energy transfers at the surface of the materials is thought to be the basis for this unique ‘reagentless’ photonic phenomenon. The emitted light continues to increase in intensity as more biological agents contact the Bio-Alloy™. This unique intensity shift of the activated Bio-Alloy™ forms the basis for a real-time biological agent detection and identification.

Advantages cited by company personnel regarding this technology are:

- Since Bio-Alloy™ smart materials can be readily adapted with different recognition elements, biological arrays of different Bio-Alloy™ materials could be placed in micro-arrays that would be spatially excited with a laser or LED. A light intensity shift in any

location would indicate the presence, identity and possibly even the concentration of a specific agent.

- Bio-Alloy™ is highly versatile; it could be placed in arrays on integrated circuits, used to coat fiber optic wave-guides or stacked in capillary columns.
- There is potential for lateral translation of the core Bio-Alloy™ platform technology into other sectors (e.g., civil defence, medical diagnostics, environmental monitoring, etc). Bio-Alloy™ technology allows for an almost universal detection system to be developed. The core technology will readily evolve with other biotechnological recognition element developments.
- Although it would be possible to clean and reuse Bio-Alloy™ once it had alarmed, due to its potentially very low cost, the best solution might be to incorporate it in a canister and "advance the film" like a camera to expose a new region.
- Since Bio-Alloy™ "holds" the bound biological agent, an activated segment can be used for forensic analysis and further confirmatory testing.
- It is theoretically possible to manufacture 10s, 100s or even 1000s of unique "spots" of Bio-Alloy™ materials in a micro-array format. This would allow confirmation of exposure to one or more agents at a time. It would also allow placing variants of recognition elements in a single array to identify the sub-strain of the biological agent (e.g. which of several variants of "anthrax" is being used).
- In its current form, Bio-Alloy™ is working at the level of the most sensitive immunoassays even with the existing optical hardware only capturing 1% of the emitted light. They envision that the next generation miniaturized system should capture greater than 10% of the emitted light, leading to an even more sensitive system.
- The light emitted from activated Bio-Alloy™ increases in intensity as additional bio agents bind to the recognition elements. It may be possible to calibrate a system to provide quantitative (i.e. concentration) information in addition to detection and identification.
- Although the receptor binding process requires the presence of a very finite amount of hydration, a molecular film around the receptors is all that is required. Bio-Alloy™ is fully functional from 4 to 45 degrees Celsius and it could be freeze dried in sealed packages for almost limitless life span. Once opened and exposed to the environment, it is anticipated to remain functional for over three months. If incorporated into an advancing canister arrangement, the operational life could be quite long. Many current detectors require servicing every 12 hours.
- Some of the most deadly materials are biological toxins, lethal at extremely low concentrations. Although you can scan for the presence of microbiological (i.e. cell-based) agents by bioluminescence, bio-toxins such as ricin, botulinum toxin and others will not luminesce. Bio-Alloy™ materials with the proper recognition elements have the potential to detect and identify these agents along with a wide range of microbiological agents.
- Bio-Alloy™ technology is compatible with wet or dry sampling systems. Liquid samples are easier to handle than gas since simple capillary action can be used to draw samples across the sensing materials. An air phase system is more complex because air must be concentrated and entrained in the finite liquid phase on the smart materials. Thin channel laminar flow of air or liquid samples in such a design would tend to be self-cleaning yielding a low fouling process.

However, there are some concerns with this technology. The only testing to date has been carried out with simulants, under controlled conditions. However, due to the similar nature of the underlying binding of receptors with such simulants in comparison with 'live' agents, this suggests that it will be applicable for BW agent detection. The technology remains to be tested under "real world" conditions (dust, humidity, background contamination, diesel exhaust, etc) or under "soldier" conditions (handling, transport, etc). IatroQuest plans to conduct 'live' agent testing at DRES in the next year. This testing will be undertaken in controlled chambers. It was unclear whether IatroQuest has a "front end" identified for air sampling for their

system. Due to the detection sensitivity of the technology to recognize the presence of an agent, the volumes of air required may not be high for effective detection to take place. Despite this, the issues of collection and concentration have not been resolved which will likely be an integral part of the company's strategic partnering.

IatroQuest representatives envision that this technology could eventually lead to a future generation of pocket-sized CB warfare detector. The resulting devices, enabled by the smart materials, would be designed to complement or replace the much larger and more expensive instruments used by the U.S. military. All lethal CB agents have molecular "flags," or signatures, which identify them and the "fuzzy logic" program in a detection device could be programmed to distinguish between common, non-fatal agents and potential killers. A real-time spectral analysis of the light by the device's palm-sized computer would reveal what agent(s) is/are present. That information could be flashed on a monitor screen, along with instructions on what to do or linked by wireless technology into the command and control network. The technology would be compatible with remote sensing as well. The sensors, after detecting the agents, would then alert the network to their presence, and indicate by collective analysis the direction of the 'cloud'. Company representatives stated that they anticipate the Bio-Alloy™ enabled devices would work even as new CB agents are developed since it would be difficult to hide all the identifying molecular 'flags'.

### *ID Biomedical Corporation*
### *Vancouver, British Columbia, Canada*

#### *Company Overview*

ID Biomedical Corporation is a North American biotechnology company established in 1991. Their headquarters is located in Vancouver, Canada, and the company also has a R&D facility in Bothell, Washington, and a satellite office in San Diego, California. ID Biomedical is developing products in the field of disease control: gene-based diagnostic testing and subunit vaccines. Their products in development target drug-resistant bacteria, tuberculosis, group A streptococcal disease, HIV/AIDS and *E. coli*. Part of their focus is to develop vaccines and diagnostic tests for these and similar diseases. Using their proprietary Cycling Probe™ Technology (CPT), they have developed a gene-based testing platform called Velogene™. Company representatives believe that, with further development, the Velogene™ platform will be suitable for use in a wide range of products that will identify diseases more quickly, more simply and at a lower cost than existing tests. Their focus for the CPT technology is twofold: in the diagnostic test or "kit" business, where they have already begun to develop products, and in the genomics industry.

In 1999, the company received permission from the U.S. Food and Drug Administration (FDA) to begin human studies for a vaccine for Group A Streptococcus. They also received FDA marketing clearance for their Velogene Rapid MRSA identification assay.

CPT has demonstrated its ability to detect disease causing bacteria from culture media as evidenced by the Velogene™ Rapid MRSA and VRE tests. Company representatives believe that other culture-based diagnostic tests may also be developed based on CPT but these will be "niche" products which do not allow the company to explore the broad utility of CPT in diagnostics, where technologies must be able to identify small numbers of organisms in a biological sample (such as blood). For these applications, they envision that CPT may need to be combined with other, complementary technologies. The company has made the decision not to advance CPT to this required performance level because of the significant financial and human resources required and the unpredictable nature of technology development in terms of time and outcome. They have decided to license CPT to the diagnostic industry for further technology and product development. The company has entered into agreements with Alexon-Trend and Mitsubishi Chemical Corporation in which both companies took licenses to CPT for product development in specific fields.

In addition, company product developers think CPT has some characteristics that may make it ideally suited to the rapidly growing field of genomics. They feel, though, that it is unlikely that CPT, as a stand-alone technology, will become a platform in this arena. In combination with other genomic technologies, however, CPT may play an important role. As a result, ID Biomedical expects over the course of the next year to explore business development relationships with one or more companies that could advance CPT in this new field. Already, Applied Biosystems Group has taken a broad, non-exclusive license to CPT. DiscoveRx has an option to a non-exclusive license to CPT with terms identical to those of the Applied Biosystems Group agreement. Finally, Third Wave Technologies has also acquired a license to use CPT in a narrow field covered by their Invader assay.

The Company has 29 employees. There are 25.6M shares outstanding and $C39M cash on hand. Their burn rate is $C750K per month. They are listed on the Nasdaq National Market and The Toronto Stock Exchange. The average daily trading volume is 265,520 shares. Their Q2/2000 earnings were $C0.09 per share.

#### *Technology Development*

ID Biomedical has developed a novel method for the detection of the *mecA* gene that confers the principle mechanism of methicillin resistance in Staphylococcus aureus. CPT is a rapid, isothermal method for the detection of specific target sequences. CPT utilizes a chimeric DNA-RNA-DNA probe sequence that provides an RNase H sensitive scissile link when hybridized to a complementary target DNA sequence. In the presence of target DNA, the cycling reaction converts a full-length chimeric probe into cleaved probe fragments, which accumulate and are quantified. A cycling probe designed for detection of a specific sequence with the *mecA* gene was used to develop a culture confirmation

assay for methicillin resistant *Staphylococcus aureus*. The CPT assay was used to screen 823 *S. aureus* isolates and the results were in agreement with detection of the *mecA* gene PCR.

ID Biomedical is currently advancing their lead product, StreptAvax™, in human testing. Human testing began in October, 1999 at the University of Maryland's Center for Vaccine Development. In February, 2000, they received results from the low dose study, which showed that at a dose of 50 micrograms of StreptAvax™ was safe and well tolerated. Additionally, all subjects developed an antibody response to StreptAvax™, which is a prerequisite for the vaccine's ability to prevent disease caused by group A streptococcus. Based on these results, the FDA determined that StreptAvax™ may proceed in human testing at a dose of 100 micrograms. StreptAvax™ is the only group A streptococcus vaccine cleared by the FDA for human testing and the only group A streptococcus vaccine currently sponsored and funded by the U.S. National Institutes of Health in human testing.

The DRES, in collaboration with the Department of Chemistry, University of Alberta, and the Alberta Micrelectronics Corporation, are reviewing the potential for developing an automated microchip-based platform that employs CPT for gene probe assays. Electroosmotic pumping and capillary electrophoresis are used for fluid transport and separation. Gene probe assays were carried out using CPT. Design for the on-chip CPT processor incorporates injection, nucleic acid hybridization, enzyme cleavage and CE separation and detection. The CE separation of nucleic acid probes and fragments was carried out in a nongel format.

Gene based products ID Biomedical has in development (not yet cleared or available for sale) are :

- **Velogene™ Rapid VRE Assay** is a rapid gene based test for the identification of vancomycin resistant Enterococcus (VRE). Enterococcus faecalis and Enterococcus faecium isolates that are resistant to vancomycin (an antimicrobial agent) are part of the normal gastrointestinal flora of humans, but they can cause serious infections such as bacterial endocarditis and bacteriemia.
- **Velogene™ Rapid MTB Assay** is a rapid gene based test for the identification of tuberculosis in humans.

Vaccine products in development by ID Biomedical (not yet approved or available for sale) include:

- Group A Streptococcus (GAS) vaccine is a sub-unit vaccine being developed to prevent GAS infections. GAS causes a wide variety of diseases including "strep throat" (acute pharyngitis), impetigo (a skin infection), invasive fasciitis (also known as flesh-eating disease), toxic shock syndrome and rheumatic fever. Their GAS vaccine is the subject of a human clinical trial partnership with the National Institutes of Health.
- HIV/AIDS vaccine is a subunit vaccine being developed to prevent or delay the onset of AIDS in people infected with HIV. The possibility of using the vaccine to prevent HIV infection is also being investigated. The National Institutes of Health is providing research funding to the company's collaborator, Dr. Arye Rubinstein of the Albert Einstein College of Medicine.
- Tuberculosis (TB) vaccine is a subunit vaccine being developed to prevent the onset of TB. Their vaccine is being developed with Pasteur Mérieux, Sérums and Vaccins S.A. (a member of the Rhône-Poulenc Group, a major vaccine company).
- *E. coli* vaccine is a subunit vaccine being developed to prevent *E. coli* infection. The project will initially target enterohemorragic *E. coli* and enteropathogenic *E. coli*. They are collaborating with the University of British Columbia on a R&D project aimed at developing new vaccines for *E. coli* infection.

### *Idaho Technology, Inc.*
### *Salt Lake City, Utah*

### *Company Overview*

Idaho Technology (IT) Inc., located in Salt Lake City, Utah, was incorporated in 1990. Through a university / industry partnership, IT developed the first instruments engineered to match the speed of biochemical reactions using hot air, creating the Air Thermo-Cycler and RapidCycler. IT learned that heating and cooling the samples with blasts of high velocity air results in nearly instantaneous temperature transactions, in minutes instead of hours. This break through technology developed an entirely new approach to rapid testing of DNA-based samples.

In 1996, under a National Institute of Health STTR grant, IT launched the LightCycler, an ultra-rapid thermal cycler with a built in fluorimetric detection system, for real-time on-line quantification of DNA samples. The LightCycler allowed users to complete typical PCR experiments and analyze the results, in less than 30 minutes.

Advantages of the product soon attracted the attention and investment from Roche Molecular Biochemicals, a division of Roche Diagnostics and the world's leading Diagnostics Company, which licensed the technology from IT in 1997. This relationship proves the soundness of IT's original vision and made rapid thermal cycling the new standard world-wide.

IT has also been awarded two more STTR grants for development of homogeneous multiplex PCR by fluorescence and temperature of melting and high resolution melting curve analysis. In addition to the current SBIR, they are working under from National Institute of Health, to develop real-time quantification with internal standards.

Some of the marketplace uses they are targeting for their systems are:

- On-line quality control of food manufacturing
- Water infrastructure protection
- Point-of-care diagnostics coupled with global epidemiology.

### *Technology Development*

In 1998, IT completed a contract with the USAF for the Ruggedized Advanced Pathogen Identification Device (RAPID), a field-hardened rapid thermal cycler with real-time on-line monitoring. The RAPID is capable of automatically analyzing samples for the presence of any nucleic acid sequence, in both laboratory and austere testing conditions. An advanced artificial intelligence system allows the RAPID to automatically collect the data, interpret the test data, and report the results, providing the capability for biological pathogen testing and identification in the field. The system integrates IT's LightCycler technology into a portable, rugged package with software that allows "push button" use by field personnel with minimal training. Field personnel can then prepare samples, place them in the instrument, and push one button. The RAPID then runs the appropriate reaction, analyzes the flourescence change in the samples, and displays the results.

RAPID also provides a real-time reach-back capability that allows real-time monitoring of the reaction process from a remote location via a standard or secure Internet web browser. This permits reactions that are run in the field to be monitored by experts located anywhere an Internet terminal is available.

The diagnostic detector was used in Saudi Arabia, when U.S. troops stationed there reported serious diarrhea and gastrointestinal distress, for which salmonella was determined to be the cause. The outbreak was contained to three percent of the soldier population. Company representatives envision that this detector can also be used for civilian purposes as well, such as by food manufacturers, hospitals, fire departments, and police stations.

The company is working to expand upon this technology in order to fully automate BW identification systems. They are researching this in conjunction with many DoD customers. Included in these efforts are assay testing kits made under ISO 9001 conditions, continuously monitoring detection devices and smaller, faster versions of the RAPID.

In conjunction with the USAF, EYT and ORACLE®, IT is also promoting LEADER, a large-scale surveillance system. LEADER will provide a macro-level view and analysis of

force-wide reporting of biological events. This would be extremely useful in assessing and responding to a wide range of biological incidents at the national level. LEADER takes data analysis and brings it to the level of knowledge distribution, necessary for command and control.

### *IGEN International*
### *Gaithersburg, Maryland*

#### *Company Overview*

IGEN International, located in Gaithersburg, Maryland, is a medical products company that develops, manufactures and markets diagnostic systems utilizing its patented ORIGEN technology, a universal diagnostic platform that addresses many segments of the industry. The company and its collaborators design ORIGEN-based diagnostic systems for multiple segments of the diagnostic market in three principle areas: the clinical diagnostic market, including central hospital laboratories, clinical reference laboratories, patient point-of-care testing and home testing; the life science market, including laboratories in pharmaceutical and biotechnology companies, universities, private institutions and the government; and the industrial market, including food and water quality assurance programs, and animal health testing.

The company is focusing on being able to deliver heightened sensitivity to various assays involving nucleic acid amplification such as PCR for use in such research fields as gene sequencing and by researchers that need to label proteins such as in receptor-ligand binding studies, or conventional inummoassays. IGEN has strategic alliances with Bochringer Mannheim, GmbH (Mannheim, Germany), Eisai Co., Ltd. (Tokyo, Japan) and Organon Teknika (Oss, The Netherlands) to develop new analytical tools based on ECL technology. Perkin-Elmer (Norwalk, Connecticut) has licensed the ECL technology from IGEN and has made the system commercially available as the QPCR-500O$^{Tm}$.

Clinical inummoassays developed using this system include cancer markers such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP) and prostate-specific antigen (PSA); hormones such as thyroid stimulating hormone (TSH); therapeutic drugs such as digoxin and theophyline; and infectious diseases such as hepatitis B surface antigen. IGEN is developing miniaturized instruments which use micro-disposable electrodes for clinical immunoassays that can be used for near patient testing - point-of-care testing.

Nucleic acid hybridization based assays, similar to the formats described for immunoassays, have also been developed to detect HIV, cystic fibrosis, and human papilloma virus. Nucleic acids were amplified using the PCR or Nucleic Acid Sequence Based Amplification (NASBA).

#### *Technology Development*

IGEN developed their ECL detection technology for applications in diagnostics, drug discovery and development, and the basic research laboratory. ECL is a detection method that is stable in air and aqueous solutions. It is capable of quantitating the binding of any two molecules that come together with specificity. IGEN representatives have stated that they expect to achieve significant enhancements in inummoassay performance in areas such as sensitivity, dynamic range, assay kinetics and format flexibility.

ECL is the process by which light generation occurs when a low voltage is applied to an electrode, triggering a cyclical oxidation and reduction reaction of a ruthenium metal ion. The ruthenium ion is bound in a chelate of tris-(bipyridine). A second reaction component, tripropylamine (TPA), present in the assay buffer in vast molar excess, is consumed in the oxidation process and the ruthenium chelate is recycled. The labeled component is captured on the surface of para-magnetic beads which are brought to the surface of an electrode by a magnet; the second oxidation reaction component, TPA, is introduced into the flow cell and a voltage is applied. This association with the electrode results in a very fast electron transfer reaction. The transfer initiates the excitation of a reporter molecule that is also in close association with the electrode which ultimately results in emission of a photon of light at a specific wavelength. The voltage oxidizes both the ruthenium and the TPA simultaneously. By losing a proton, the TPA becomes a reducing reagent that transfers an electron to the ruthenium ion. The electron joins the ruthenium in an excited state and it then decays to the ground state, releasing a photon in the process. Unlike the TPA, which is consumed in the process, the reduced ruthenium ion is recycled and therefore continues to produce light. The TPA, present in vast molar excess, and the unique, recycled ruthenium molecule, together intensify and amplify the ECL signal.

The heart of IGEN's ECL-analyzer system is an electrochemical flow cell with a photomultiplier tube placed just above the working electrode for efficient light detection. In order to deliver the molecule of interest to the electrode surface, magnetic bead tagging has been implemented into the system's design. Under the working electrode, a magnet is positioned for either capture or release of the beads coated with target molecules. Automated sample handling equipment and a fluidics delivery system round out the system.

A typical read cycle begins after addition of the beads to the reaction mixture in 1205mm tubes. The tubes are placed in a vortexing carousel to keep the beads in suspension prior to sampling. An automatic sample delivery system aspirates a user-determined volume (175 to 1000 µL) of the total reaction mixture and pumps it into a flow cell. As the sample is pumped through the cell, the beads are captured by a magnet onto a platinum electrode. The electrode is charged with less than two volts, which triggers light production from the oxidation reactions. In less than one second, all the light emitted is measured in a photomultipher tube and digitally stored. The cell is then washed with a cleaning buffer in preparation for the next reading.

In a typical immunoassay, anti-target antibodies are bound to magnetic beads. Next, anti-target antibodies recognizing a different epitope on the same target are made into reporter molecules by attaching an ECL label. Incubating the target molecule with both antibodies results in formation of a "sandwich" - the two antibodies attaching to the antigen at different sites. As this "sandwich" sample is being drawn into the cell and mixed with a buffer solution containing precursors, an applied magnetic force will capture the magnetic beads on the electrode surface - stabilizing the target molecules and their attached reporters for maximum detection by the PMT. Unbound reagents from the sample mixture are washed away by continued flow of assay buffer solution. After sample capture, the pump is stopped and ECL measurement is performed by application of a potential perturbation to the working electrode. This gives an extremely clean signal to noise ratio. Only those labels bound to the "sandwich" surrounded by precursors and in contact with the electrode emit light with little interference from the buffer background. After measurement the magnet is released, a cleaning solution is drawn into the system to flush it, then more precursor containing solution is pumped into the flow cell to flush the cleaner.

IGEN representatives assert the improved assay performance offered by ECL over traditional RIA or ELISA is substantial. They indicated that the no wash format means reduced labor, improved intra and inter-assay precision (typically 5%-8% or less), and waste problems. The increase in kinetics over ELISA's means faster assays. The increase in sensitivity allows at least a reduction in sample volume or rare reagents and possibly the opportunity to do assays that were otherwise not possible. Because the user does not handle the sample after assay incubation, there is an improvement in precision. The requirement for extensive washing to lower background using other detection technologies restricts the quantitation of antibody or receptor binding when the affinities are in the micromolar range or less. As the necessary washing occurs for traditional assays, low affinity reagents begin to disassociate, leading to poor assay performance. The removal of required wash steps, and rapid quantitation after incubation provides good quantitation of low affinity binding in the ORIGEN instrument. The ORIGEN System includes both the ORIGEN Analyzer to read samples, as well as an IBM compatible computer with software developed to run the instrument.

A number of leading pharmaceutical companies have implemented IGEN's technology in drug discovery research and are now using it in the preclinical and clinical settings. IGEN representatives noted that the ECL technology can provide more than a positive or negative response in primary drug screening. The potential of acquiring a quantitative spectrum of information of drug activity between the traditional negative and positive signals generated in the drug screening process will produce more information on new compounds and related families of compounds. They noted that the technology is also well suited for rapid quality assurance/quality control analysis and fermentation monitoring as well as on-site equipment and product validation.

### *Johns Hopkins Applied Physics Laboratory*
### *Laurel, Maryland*

#### *Laboratory Overview*

The Applied Physics Laboratory (APL), a not-for-profit research division of The Johns Hopkins University, supports the DoD and other Government agencies through applied research and technical development. Located in Laurel, Maryland, the Laboratory employs approximately 2,700 engineers, scientists, and supporting staff. The lab maintains 90 specialized research and test facilities. Major sponsors include the U.S. Navy, U.S. Army, USAF, NASA, DARPA, and the Departments of Transportation, Energy, and Treasury. The lab's average annual funding level is $420M through more than 200 separate tasks.

The Milton S. Eisenhower Research and Technology Development Center (RTDC), one of APL's technical departments, is dedicated to basic and applied research in support of APL's mission. The RTDC contains a number of specialized laboratories and conducts research programs in such areas as sensor science and technology; system and information sciences; physics, modeling, and applications; and aeronautical science and technology.

In its sensor work, the laboratory is designing MS-based approaches to identifying microorganisms for application in such areas as clinical medicine and medical research, environmental monitoring, law enforcement and defense. Doctors would use this capability for quick analysis of cultured organisms. Environmental monitoring personnel could use the device for analyzing CB substances in the water and air for pollutants and toxic substances. Security personnel would be able to identify volatile solvents used in drug laboratories, drug breakdown products, and arson initiators. And military personnel could use the detector to detect CB warfare agents on the battlefield or during treaty verification.

#### *Technology Development*

APL's Milton S. Eisenhower RTDC focus is on developing new classes of sensors while miniaturizing and extending the performance of existing sensors. Decreasing the size of sensors while improving capabilities is a major RTDC focus. Among the instruments under development at APL are:

- novel miniature magnetometers
- a solid-state optical-sensor that serves as a platform for a host of CB sensors in either gaseous or liquid environments
- a single handheld, automatic electronic device, the Fluorometric Affinity Biosensor, that replaces large, cumbersome, chemical laboratory analysis systems.

APL, in conjunction with the Johns Hopkins University Medical Institution and the University of Maryland, Baltimore County, is developing a miniaturized time-of-flight (TOF) mass spectrometer to detect trace quantities of high molecular weight CB compounds. Using the MS technology to identify substances that form during vaporization and ionization, the tiny TOF instrument will fit in a pack about the size of a shoe box and will be used as a portable universal sensor for analyzing solids, liquids, or gases in the field. In the TOF mass spectrometer, molecules are broken down into ions and molecular fragments on a high voltage surface. Fragments then drift through a vacuum, with drift speed depending on fragment mass. Fragments of different sizes arrive at the detector at different times and are analyzed separately as they arrive.

A single mass spectrometer can generally identify a pure substance unambiguously. Samples containing many compounds require two analyzers working in tandem. The miniature mass spectrometer uses an ion reflectron device to extend the drift region. A single small instrument could provide tandem capabilities in a 12x6x6 inch package weighing less than 11 pounds and requiring less than 50 watts to power.

APL researchers stated that work on the tiny TOF measuring concentrations of chemicals and biochemicals for military and civilian applications has led to further reductions in instrument size and to improved performance. The instrument incorporates signal processing, high-speed digitizing electronics, fast pulsed lasers, and miniaturized vacuum pump designs. Researchers state that the system also uses new techniques for ion formation and energy-focusing, new sampling and ionization schemes, and new analysis techniques.

### *Lincoln Laboratory, Massachusetts Institute of Technology Lexington, Massachusetts*

### *Laboratory Overview*

The mission of Lincoln Laboratory, established in 1951 in Lexington, Massachusetts as a Federally Funded R&D Center of the Massachusetts Institute of Technology, is to apply science and advanced technology to critical problems of national security. The scope of the problems has broadened from the initial emphasis on air defense to include communications, space surveillance, air traffic control, and environmental monitoring. Throughout its history, the Laboratory has had an extensive program in advanced electronics technology. Their core competencies include sensor technologies, complex systems, field measurements, sensor nets and algorithms, operational systems, and meteorology.

In pursuit of its mission, Lincoln Laboratory activities include:

- Evolution and demonstration of feasibility of advanced system concepts and technology
- Specific programs of R&D, including the building of necessary components
- Construction of initial models of laboratory-developed equipment for field demonstration

Lincoln Laboratory works under an omnibus contract to USAF/ESC (Electronic System Command) at Hanscom AFB, and is funded via Military Interdepartmental Purchase Request and periodic contract modification. They receive funding from the USAF, U.S. Army, U.S. Navy, DARPA, other DoD agencies, and various non-DoD agencies.

Lincoln Laboratory has spun off more than 65 companies that employ 136,000 people and generates over $15B in sales; the laboratory has been granted 416 patents, 276 of which are licensed. Lincoln Laboratory enters into CRADA arrangements and licensing agreements with industry to aid in transferring technology.

### *Technology Development*

Lincoln Laboratory initiated work in the BW defense arena five years ago. Their program has grown to $12M in FY 2000. Lincoln Laboratory has a number of different research efforts ongoing in the BW defense area. Components of their biological defense program include:

- BW threat analysis
- Stand-off system performance analysis
- Defensive system requirements
- Networked sensor solutions
- UV-LIF sensor development
- Lab measurements of sensor performance/sensor improvements
- Environmental background measurements
- Technology transfer, including JBPDS insertion
- Intelligent particle sampler
- Agent preparation from environmental samples
- Nucleic-acid based bio-agent identification
- Sensor modeling.

One effort, now completed, involves developing a DNA sequencing method 100x faster than previous methods. Leveraging a multi-year National Institute of Standards and Technology supported genosensor development program, Lincoln Laboratory has teamed with seven industry partners to design this new approach. The team has completed a first-generation system prototype that is now being developed within the biotech industry.

Lincoln Laboratory is developing BAWS, which began in 1996 as part of an U.S. Army ATD on point detection technology for early warning of a biological agent attack. Three sensor generations were developed in the course of 3.5 years. The sensors have undergone testing both at Dugway Proving Ground and at other locations. Lincoln Laboratory is presently integrating BAWS in the Joint Point Biological Detection System (sponsored by the JPO-BD), which includes an integrated suite of sensors to detect and identify a biological attack. They are working in conjunction with Battelle and Lockheed Martin in this development activity, designed to replace the particle counter (trigger) and flow cytometer (detector) with this technology. Their goal is to improve performance and reduce life cycle costs. They hope to achieve these goals by improving sensitivity in highly stressing backgrounds,

reducing prime power consumption, and decreasing size and weight. The first of these systems is scheduled to be fielded by second quarter 2002.

Lincoln Laboratory also provided an eight-month assessment of the SR-BSDS built by Fibertek to the JPO-BD. They applied BAWS-III biological-discrimination algorithms to SR-BSDS cloud return data and analyzed concepts to enhance signal-to-noise-ratio, reduce false alarms, and increase early warning capability. In conjunction with this activity, Lincoln Laboratory is evaluating the capability of UV-lidar to provide bio-discrimination at significant stand-off distances. A team of scientists is analyzing prototype SR-BSDS laser return data and applying the BAWS-III point sensor bio-discrimination approach to establish a method for early detection of bio-agent release.

In addition, Lincoln Laboratory has developed an immune-based biological agent identification sensor – the CANARY sensor. By utilizing established immunoassays and gene-insertion technologies, scientists from Lincoln Laboratory used B cells extracted from mice to develop this approach, which the scientists have stated will offer signal amplification , speed and possible single-particle identification, while being resistant to contamination. The rationale for this technology is that some biological agents can infect at the 1-10 particle level. A biological particle detector without a fast, sensitive identification capability could not detect a low-level attack in the presence of typical biological background counts. The Lincoln Laboratory scientists believe this new cell-based sensor concept provides the capability to respond in less than 1 minute and offers high sensitivity. Different B cells can be targeted to specifically identify different biological agents. The design is suitable for simultaneous multi-agent detection and should be able to detect and identify a two particle/liter cloud in less than 1.2 minutes. Remaining work on the program includes genetic engineering of cell lines for specific biological agents and construction and testing of a field prototype. They are working in conjunction with CDC, NMRC, USAMRIID, and the U.S. Army to develop and test.

### *Majesco Biologicals, Inc.*
### *Edison, New Jersey*

#### *Company Overview*

Majesco Biologicals, Inc. (MBI), located in Edison, New Jersey, provides rapid immunoassays test kits for detecting microbial pathogens and technical and operational support for end-users.

MBI was formed as a medical diagnostics company (Majesco Medical Technologies, 1993) and was one of the early companies to develop low-cost, simple diagnostic test kits for the rapid detection of various infectious diseases such as HIV, dengue, and cholera. This work expanded to fill the emerging need for the development and production of rapid test kits for detecting various microbial pathogens in environmental samples. MBI's corporate offices are in Edison, New Jersey with offices in Washington, DC and Baltimore, Maryland. Currently, the primary laboratory and production facility is in Princeton, New Jersey.

Through a Cooperative Research and Development Agreement (CRADA) beginning in 1996 between Arista Biologicals (Bethlehem, Pennsylvania) and the U.S. Navy Medical Research Institute (NMRI), Bethesda, Maryland, antibody-based immunochromatograhic assays (also known as 'flow-through' assays or hand held assays) were developed for detecting potential BW agents in environmental samples. MBI acquired the technology information from Arista for the co-development of these assays in 1997. Subsequently, MBI and Princeton BioMeditech, Corp. (PBM) through a joint venture agreement began manufacturing prototype, pilot-scale BW detection assays for the JPO-BD in 1997. In 1998, total production had increased to approximately 300K assays. The number of different assay types manufactured has increased to 18 in FY 2000. Included in these 18 is the six assay-training panel developed by MBI. During CY 1999 and first quarter 2000 the total production was approximately 700K individual assays delivered to the JPO-BD (see below for description of various configurations).

MBI has been the primary supplier of these BW detection test kits to the DoD. MBI representatives noted that, as a supplier, MBI is required to use the critical reagents (antibodies and antigens) approved and supplied by the JPO-BD for the manufacture and evaluation of these test kits. These reagents are produced by other producers/vendors in accordance with JPO-BD protocols and specifications and shipped to MBI as needed to manufacture various lots of assays. At this time, it is the JPO-BD's position to not permit the use of reagents from any other source (academia, industry, etc.) to manufacture these specific BW detection products.

MBI's business development has expanded beyond the typical BW terrorist scenario against humans. MBI holds a licensing agreement with the University of California - Davis for the manufacture and distribution of diagnostic products for a variety of veterinary viruses. These detection assays target the testing of animals in the field. Since the assays and supporting sampling material do not require special handling or refrigeration, they are of interest to developing countries and remote locations.

Additionally, MBI is pursuing a number of collaborations to develop next generation assays with improved sensitivity and specificity. These new or alternate technologies will be adapted for determining the presence of pathogen/agent contamination in more complex media such as food, water and other environmental samples.

#### *Technology Development*

MBI test kits are designed to provide a rapid screening tool for the presence of biological agents (bacteria, viruses and toxins). MBI spokespersons noted that, as with all screening tools, a reference laboratory should confirm the presumptive field analyses. The results of these screening tools can assist the reference laboratory in quickly confirming the presence of a biological agent.

The test kits utilize technology that includes specific production strategies developed during Arista/MBI's work on the U.S. Navy CRADA, patented technology available to PBM, and other strip production trade secrets and know-how contributed by the MBI/PBM joint venture.

MBI manufactures the needed immunoassays as individual strips that have been configured into various formats for use by the JPO-BD in the following detection systems:

- Hand Held Assays
- Biological Integrated Detection System (BIDS)
- Joint Biological Point Detection System (JBPDS)
- Joint Biological Remote Early Warning System (JBREWS) Advance Concept Technology Demonstration (ACTD)
- Portal Shield ACTD

Other government agencies (Federal Bureau of Investigation, Department of Justice, Health and Human Services/Center for Disease Control, etc.) and civilian first responders (fire chiefs, police, etc.) can obtain these BW detection HHAs for their own use by requesting them through the JPO-BD. At this time, MBI is not permitted to directly provide BW detection assays manufactured using government furnished reagents to any third party. Only at the direction of the JPO-BD, can MBI ship various agent test kits to Non-DoD end users. However, MBI can provide generic product samples, non-DoD assays, training kits and supporting training material to interested parties.

Military personnel or civilian first responders use these BW detection assays to provide *rapid* presumptive identification of a possible BW attack in the field (i.e., on-site). The tests are typically used as individual HHA or integrated into devices that are read by an electronic scanner. The initial field results (positive or negative) are available within 15 minuets. The advantage of using these assays on-site is to provide rapid results that can be combined with other available information to immediately guide the appropriate response efforts. It is important to note that these assays provide a preliminary determination if a BW agent could be present. A confirmatory laboratory, where more traditional and sensitive microbiological techniques are employed, must confirm the final analysis.

The basic approach to strip production involves a lateral flow immunochromatographic strip assay's having a specific arrangement of elements: a base membrane, and in linear arrangement upon this membrane there is a sample reservoir pad, a filter zone (one or more), and a wicking membrane. The base membrane normally is a plastic strip, which supports all other components. The sample reservoir pad is treated with chemicals that prepare the sample properly for the assay, and are customized for each specific assay type. The sample is added to the sample reservoir pad, from which it travels to one or more filter zones, one of which contains sprayed lines of antibody upon which the test bands are read, and finally to the wicking membrane.

Critical manufacturing aspects include:

- Preparation of the gold conjugate of the detector antibody, and determination of the appropriate strength and composition of the conjugate solution that contains this antibody/gold conjugate
- Proper spraying and blocking of the nitrocellulose membrane
- Chemical treatment of the sample reservoir pad to reduce or eliminate cross reactants, disperse or solubilize antigens or antibodies, and adjust the sample to proper ionic and pH conditions
- Assembly of the strip components, in particular the nitrocellulose membrane of the filter zone and the spraying of capture antibodies on that membrane.

Approximately 100 µl of appropriate liquid sample is applied to the sample well and allowed to develop for 15 minutes. The control line will develop at the top of the test window and if the sample is positive, the test line will develop in the bottom of the test window. The test line may develop and be visible as a purple colored line before 15 minutes (dependent on concentration). The test line may be lighter than the control line.

- Positive = Two lines in the test window. Both a control and test line
- Negative = One line in test window. Single control line
- Inconclusive = no control line (with or without a test line)

If a test result is negative at 15 minutes but appears to become positive after 15 minutes, it should not be interpreted as a positive. Testing beyond 15 minutes should not be performed.

In addition to large-scale production of BW test kits for the DoD, MBI also has a CRADA with the ECBC. This joint collaboration will look at the development of additional assays for the detection of biological material of interest to the DoD and other government organizations such as Department of Agriculture, Food and Drug

Administration, and various international organizations.

MBI representatives stated that the BW threat is not limited to attacks against people. Animals, food processing, distribution and handling systems, crops, etc. are other areas vulnerable to contamination either by a terrorist incident or natural outbreak. There is currently a limited capability in the intelligence, law enforcement or public health communities to address these potential threats in a comprehensive, but affordable, manner. MBI believes that reliable and inexpensive rapid testing devices (whose results are linked in a smart network) offer the best approach to providing near-real-time warning of such attacks against people, crops, livestock, food and water supplies until there are major technology breakthroughs in detection devices.

### *MesoSystems*
### *Richland, Washington*

#### *Company Overview*

MesoSystems Technology, Inc., located in Richland, Washington, is a two-year-old biotechnology systems small company that manufactures airborne microbial control systems for national security and public health markets. A central focus is on the development and manufacture of products for biological detection for both military and civilian counter-terrorism defense markets, as well as food, biotechnology, and medical technology markets. MesoSystems principal activities in this area have been directed toward developing technologies for the detection of biological active particles and chemicals and protection against these materials. Their primary areas of concentration are:

- pathogen collection, detection and concentration,
- CB protection (clean air and water) and
- decontamination/sterilization.

The company's key market areas are environmental sampling, air monitoring and rapid bio-detection. They recently introduced a hand-held air sampler that captures bacteria and viruses from the air and concentrates them into small liquid samples or onto a solid surface. The BioCapture™ Air Sampler has been specially designed to collect air samples in places potentially containing BW agents. The BioCapture™ Air Sampler collects airborne pathogens and enables the user to quantify concentration levels. The microbes are captured and concentrated into an aqueous sample. Whole cell rapid detection, nucleic acid or other liquid based sensor systems can then be used for analysis. A tape mechanism can also be used for more continuous processes.

Another MesoSystems product is the BioVIC™, an aerosol collector that serves as a front-end air sampler for biological detection systems. The BioVIC™ preconcentrates the air stream, capturing large numbers of particles into one of three mediums. The particles can be delivered into a small volume of liquid, into a small air stream or onto a solid surface for delivery into the user's sensor. The BioVIC™ can be used with:

- PCR
- Fluorescent based optical sensor
- MS
- Pyrolysis GC MS
- Flow Cytometry.

MesoSystems can perform fluids modeling, computer aided design, fabrication, testing, analysis, and diagnostics for a range of micro-electro-mechartical, chemical, biological, and fluidics technologies. Currently, they have laboratory space that is divided into an aerosol test facility, a plasma systems lab, and a thermal systems lab.

#### *Technology Development*

MesoSystems has R&D relationships with the U.S. Army, U.S. Navy, USAF, USMC, DOE, Special Forces and DARPA.

Their R&D efforts are focused on three technologies:

- Airborne Pathogen Collection and Concentration Systems,
- Corona Plasma Decontamination Systems, and
- Compact Thermal Systems.

Their biological-aerosol collector/concentrator effort is currently directed at the refinement and rapid commercialization of the micromachined collection and sampling technology and on providing an integrated solution to sample collection, concentration and preparation to the bio-sensor community. Company representatives have stated that MesoSystems has a suite of products and engineering capabilities established to achieve this goal. Products available include micropumps, micro-dialysis, micromixers, and the MicroVIC™ aerosol collector. In addition, they have developed a comprehensive microfluidics and microelectronics competency for system integration.

MesoSystems has designed, fabricated and tested several prototype aerosol collection systems. Company officials have stated that their use of a microsystem's design philosophy, which emphasizes a scale-up approach using parallel arrays of miniature components, has resulted in a new generation of aerosol collection technology. Their Aerosol Collectors include the Micromachined Virtual Impactor Collector and the Micromachined Radial Virtual Impactor

Collector. MesoSystems has established an aerosol testing facility for the performance assessment of their prototype micromachmed aerosol collectors.

MesoSystems officials conducted a six-month study for the U.S. Army to assess the feasibility of three different particle separation approaches to be used in an aerosol collection system for biological material. Company representatives stated that their research resulted in micro-machined devices that increase collection and concentration efficiency while reducing size, power requirements and physical damage to the biological materials gathered. The development of these prototypes forms the basis for their next phase of research to develop, demonstrate, field test and commercialize an integrated, ruggedized system.

MesoSystems Technology is focused on the development of two patented strategies for particle separation: centrifugation and virtual impaction.

MesoSystems Technology is also developing a product named the MicroCentrifuge™, which is an extremely low pressure drop air filter. The MicroCentrifuge™ is a continuous flow, self-cleaning device. It is capable of removing all particulate greater than ten micron.

Complementing their bio-detection product line are R&D efforts to develop plasma and thermo-catalytic technologies for decontamination of air and solid materials exposed to toxic CB substances. They have several DoD-funded programs. Three are aimed at decontamination of air and water, and a fourth is focused on surface sterilization.

### *Midwest Research Institute*
### *Kansas City, Missouri*

#### *Company Overview*

Midwest Research Institute (MRI) is an independent, not-for-profit contract research organization that performs basic and applied research and provides technical services to industry, government, and other private and public groups worldwide. Founded in 1944, MRI has a current annual research volume that exceeds $215M.

MRI headquarters and laboratories are located in Kansas City, Missouri, USA. Satellite research offices are located in Cary, North Carolina; Washington, DC; Mountain View, California; and Indialantic and Palm Bay, Florida. The National Renewable Energy Laboratory (NREL) in Golden, Colorado, is managed and operated by MRI for the U.S. Department of Energy.

Of the total MRI staff of 1,169 professional and support personnel, more than 370 are based in Kansas City and 64 work at satellite locations. Over 25 percent of the Kansas City and satellite-based staff have advanced degrees. Approximately 732 MRI staff are located at the NREL and are dedicated to NREL operations.

General areas of MRI expertise include:

- Chemical Sciences
- Health Sciences
- Environmental Sciences
- Applied Engineering
- Computer Sciences and Statistics
- Economics and Management Sciences
- Energy
- Technology Development
- Quality Assurance.

MRI has 2,800 square feet of laboratory space dedicated to biotechnology research. This includes:

- Two Biosafety Level Two (BSL-2) laboratories equipped to conduct molecular and microbiologic studies
- Five state-of-the art BSL-3 laboratories with bacteriology, virology, mycology and cell culture capabilities
- A Bioaerosol Test Chamber.

MRI's technology development initiatives are directed toward creating new products, processes, or services and usually involve intellectual property developed in-house or through government- sponsored research. MRI participates in a variety of commercialization activities and partnerships.

#### *Technology Development*

The SpinCon® air sampler/concentrator developed by MRI is a portable device for the collection of bioaerosols, particulate matter, and soluble vapors. These include micron-sized particles, airborne biological particulates, and semi- and nonvolatile chemical compounds. The system has been developed to address a broad range of advanced air sampling requirements, including:

- Hospitals and health care
- Airlines and airports
- Air regulation authorities
- Pharmacuetical production
- Refining and petrochemical
- Semiconductor manufacturing
- Utility power
- Waste incineration
- Laboratories.

Some of the applications for which the SpinCon® technology can be applied include indoor air quality monitoring such as heating, ventilation and air contitioning system monitoring, infectious disease investigations in public buildings, workplace exposure monitoring, clean-room monitoring, and air contamination levels in manufacturing operations.

The SpinCon® air sampler collects and concentrates in a liquid medium micron and submicron bioaerosol particles such as molds, pollen, fungi, bacteria, viruses, and bacteriophages. It has also proven successful in capturing a wide variety of compounds including low and moderate vapor-pressure chemical compounds. SpinCon® has been successful in collecting the compounds Sarin (GB), organophosphonates (DIMP and related chemicals), BZ, TNT, and acids derived from nerve agents.

According to MRI researchers, data collected by MRI from both indoor and outdoor environments show SpinCon® to be significantly better than other commercial samplers. It produces a

substantially higher concentration of the target analyte in a significantly shorter time. The SpinCon® is capable of sampling up to 800 L/min and delivers a concentrated sample of up to 15 mL of liquid. It can be operated in a batch or a continuous monitoring mode.

SpinCon's® sample output stream can be interfaced with instrumented analytical techniques and sensors providing real-time or near-real-time results. Compatible chemical techniques include MS, GC, MS/MS, IC, LC, LC/MS, GC/MS, and atomic spectroscopy. Biological techniques, such as standard culture, particle/organism counting, microscopy, immunoassay, PCR assay, and flow cytometry can also interfaced. In addition, samples may be split and analyzed by more than one technique.

***Molecular Devices Corporation***
***Sunnyvale, California***

### *Company Overview*

Molecular Devices Corporation, located in Sunnyvale, California, designs, develops, manufactures and markets proprietary, high performance, bioanalytical measurement systems, including software and consumables, designed to accelerate and improve the cost-effectiveness of the drug discovery and development process. The company develops proprietary core technologies which it incorporates into its bioanalytical systems, including MAXline™ Microplate Readers, Cytosensor® System, Fluorometric Imaging Plate Reader (FLIPR™) System and Threshold® System. The Company's systems have applications in many aspects of life science including the therapeutic development process, from drug discovery and clinical research through manufacturing and quality control.

Molecular Devices offers high-performance bioanalytical systems that possess levels of detection sensitivity that enable the analysis of high-density microplates and thereby increase throughput. These products also include, or are easily integrated with, automation equipment to further enhance throughput and allow complex assays to be performed with high efficiency. The company provides technologies for performing live cell assays in high throughput mode. They currently offer three product families that address different segments of the drug discovery market: their MAXline™ family of microplate readers and liquid handling systems, their Cell Analysis systems, which include the FLIPR™, Chemiluminescence Imaging Plate Reader (CLIPR) and Cytosensor® systems, and their Threshold™ system. Their MAXline™ family of microplate readers primarily addresses the assay development market and offers the assay development scientist seven differentiated microplate readers. Their Cell Analysis products, which include their FLIPR™ system, their CLIPR system and their Cytosensor® system, address cell-based research in the high throughput screening and lead optimization market segments. Their Threshold™ system is aimed at the biopharmaceutical manufacturing and quality control process and is used to rapidly and reproducibly detect potential contaminants with picogram level sensitivity.

Molecular Devices' Threshold™ system, which is comprised of a detection instrument and proprietary reagents, represented 5% of their total revenues in 1999. Their Threshold™ system incorporates their Light Addressable Potentiometric Sensor (LAPS) technology to quantitate a variety of biomolecules such as DNA, proteins and mRNA rapidly and accurately. Company representatives noted that the demand for systems which can quantitate contaminants in the manufacturing and quality control of bioengineered products is in response to the growing number of biopharmaceutical therapeutics both entering clinical trials and receiving regulatory approval for commercial sale. The Threshold™ system was designed for biopharmaceutical companies to help them achieve more sensitive and reproducible methods to detect contaminants in biopharmaceuticals during the manufacturing and quality control process. Company representatives stated that traditional detection methods, such as DNA hybridization, can be slow, difficult to use in a manner that provides reproducible and transferable results, and often require the use of radioactive materials for detection. The Threshold™ family of products includes a workstation, software and consumable reagent kits.

Their objective is to provide innovative bioanalytical systems and related consumable products for life sciences research. The company is focusing on the drug discovery market, providing systems that accelerate and improve the drug discovery processes of assay development, drug candidate screening and lead optimization.

Their revenues were $62M in 1999 and have increased at a compound annual rate of 25% since 1995. The company was originally incorporated in the state of California in 1983 and, in 1995, they reincorporated in the state of Delaware concurrent with their initial public offering. Kopp Investment Advisors owns about 25% of the company.

Molecular Devices' customers include pharmaceutical and biotechnology companies as well as medical centers, universities, government research laboratories and other institutions throughout the world. No single customer

accounted for more than 5% of their total 1999 revenues. In 1999, sales to customers outside the United States accounted for 39% of total revenues. Company representatives anticipate that international sales will account for an increasing percentage of revenues in the future. They expect to continue expanding their international operations.

Molecular Devices manufactures their products at their facilities in Sunnyvale, California and Norway. Their California facility is ISO 9001 certified. They manufacture their own components where they believe it adds significant value, but they rely on suppliers for the manufacture of selected components and subassemblies, which are manufactured to their specifications.

As of December 31, 1999, Molecular Devices employed 213 persons full time, including 44 in R&D, 78 in manufacturing, 68 in marketing and sales and 23 in general administration and finance. Of these employees, 36 hold Ph.D. or other advanced degrees.

### *Technology Development*

Molecular Devices typically invests 10% to 12% of their revenues in R&D. Over 70% of their revenues in 1999 were derived from products that the company introduced in the last three years. Their R&D expenditures were approximately $7.363M in 1999, $5.686M in 1998 and $4.721M in 1997. Their R&D activities are focused on broadening their technology solutions, including development of new proprietary reagent kits; providing more sensitive quantitative evaluation of biological events; providing greater throughput capability, especially with smaller sample volumes; and developing increasingly sophisticated data management and analysis capability.

Their MAXline™ products, which represented 57% of total revenues in 1999, consist primarily of advanced microplate readers. Company representatives noted that microplate readers have become one of the most fundamental tools used in life sciences research by addressing the increasing need for the acquisition and processing of large quantities of biochemical and biological data. They explained that microplate readers provide scientists the benefit of high throughput analysis in a standardized, multi-sample format. Because of the productivity gains using a multi-sample format, microplates have largely replaced test tubes and cuvettes for many life sciences applications.

A microplate is a disposable plastic vessel that is used with a microplate reader to measure light. The basic principles of microplate readers are that light from an appropriate source is directed to a wavelength selection device, such as a monochromator, and its intensity is measured before and after passing through each of the sample wells of a microplate. Application of a mathematical formula to the light intensity measurements of each microplate well provides a measure of the sample present in the well. The measurement, known as optical density, relative fluorescence, or luminescence, is proportional to the concentration of the substance that is being measured. Historically, the standard microplate was comprised of 96 individual wells. As cost and throughput have become increasingly important, however, the industry has begun to move to higher density plates, including 384 wells and 1536 wells. Molecular Devices representatives believe that this trend towards miniaturization will continue to be a significant factor affecting the microplate reader market in the future. The company's MAXline™ strategy has been to continue to introduce new products that include first-of-a-kind features, as well as to offer varying feature sets and price points to address different market segments. They have historically focused on the premium end of the microplate reader market through offering products with advanced capabilities.

The company acquired a line of liquid handling systems, primarily washers, through their acquisition of Skatron in 1999. Washers are used to dispense and remove fluid from microwell plates and are used as an integral step during the course of many assays.

Molecular Devices' Cell Analysis systems, which represented 38% of their total revenues in 1999, are used to study the response of live cells to drug candidates and are primarily targeted toward high throughput screening and lead optimization. Company representatives explained that many therapeutic drugs are targeted to cell membrane receptors: special proteins that function as control switches for cell activity and are triggered by the specific binding of soluble natural substances to relay messages to the cell via "signal transduction" mechanisms. Therapeutic drugs which act on receptors either

mimic or block the action of the natural receptor-specific substance. The therapeutic potential of such drugs is, therefore, most appropriately studied using live cell systems. The company focuses on providing complementary tools for studying the response of live cells to different compounds, both for research and for drug candidate screening purposes.

Their FLIPR™ system provides pharmaceutical companies with the ability for live cell analysis at a high throughput rate. Over 70 customers have purchased their FLIPR™ systems since its introduction in 1996, including the 20 largest pharmaceutical companies, several of which own five or more FLIPR™ systems. The primary applications for their FLIPR™ system are the measurement of intracellular calcium ion flux and membrane potential change, both of which provide critical information on the activation of cells by test compounds. In their FLIPR™ system, cells, along with appropriate fluorescent dyes, are maintained in microplates in a humidified, thermally-controlled compartment together with compound-addition plates. A laser light is then passed through the wells to provide excitation illumination and fluorescence from cells on the bottom of the wells. During the reading cycle, a built-in pipettor transfers compound samples from the compound-addition plate to the cell plate and the reaction is continuously monitored by an ultrasensitive charge coupled device camera, at intervals of less than one second. This strategy allows for real-time monitoring of cells before and after compound addition, thus allowing the measurement of rapid non-linear, response kinetics. Their FLIPR™ system's limited depth-of-field fluorometry optical design is patented.

Molecular Devices' CLIPR system was developed based on telecentric lens luminometer technology licensed from Affymax Research Institute in 1998. They introduced this system in the third quarter of 1999. It provides pharmaceutical companies with the ability for live cell analysis at an ultra high throughput rate using luminescence technology. Their CLIPR can support the analysis of over 200,000 samples in an eight-hour day. The system combines a charged coupled device camera with proprietary wide field optics to achieve ultra high throughput by simultaneously imaging all of the wells on a microplate. Company representatives stated that, as a result of the simultaneous imaging, CLIPR is compatible with any microwell plate format including 96, 384, 1536 and beyond. Their CLIPR system can be integrated with a linear track robot, used in workstation mode with an optional light-tight plate stacker module, or used in a stand-alone mode. The primary applications for CLIPR are cell-based and non-cell-based assays, such as reporter gene and SPA assays.

Company representatives stated that Molecular Devices developed the Cytosensor® system to provide a fast, reliable, single assay system to investigate multiple cellular functions in numerous cell types. Their Cytosensor® system incorporates their core Light Addressable Potentiometric Sensor, or LAPS, technology, a detection system capable of measuring a wide variety of chemical reactions as they occur on the surface of a silicon based sensor, into a patented system that permits researchers to conduct microphysiometry (the study of cellular metabolism) without destroying the cells. They indicated that cellular metabolism is the most fundamental and essential of all physiological processes, and allows for the monitoring of cell activation, stimulation, growth, toxicity and other biochemical events crucial to the development of new therapeutics. They believe that the primary applications of the Cytosensor® system are receptor characterization, orphan receptor identification, human cell pharmacological profiling and in vitro toxicology. The company offers a 4-chamber Cytosensor® system targeting customers with relatively low throughput requirements and an 8-chamber system for customers who require higher throughput.

Molecular Devices is expanding their reagent business by focusing on the internal development of proprietary reagent kits optimized for their Cell Analysis instruments. They have developed and produced reagent kits for their Threshold™ systems, and they recently began to sell consumables for their installed base of Cell Analysis instruments with the introduction of two kits for performing assays on FLIPR™ and CLIPR. During 1999, they hired a reagent development and marketing team, built organic chemistry labs and expanded their reagent production capacity.

### *Orbital Sciences Corporation*
### *Pomona, California*

### *Company Overview*

Orbital Sciences Corporation, located in Pomona, California, is involved in low-cost space technologies and satellite-network services. Founded in 1982, Orbital has annual sales of approximately $1B and a work force of over 5000 people. The core technologies of their space and ground infrastructure systems include satellites, launch vehicles, sensors and electronics, and satellite ground stations. Their Magellan subsidiary is a satellite access products company, producing GPS navigation and satellite communications products. Through their ORBCOMM and ORNIMAGE affiliates and ORBNAV subsidiary, Orbital provides distributed satellite networks that offer data communications, digital imagery and automotive information services.

Orbital's Sensor Systems division designs, develops and manufactures analytical instruments for space, military and industrial use. Orbital obtained this capability through the 1993 acquisition of the Applied Science Operation of the Perkin-Elmer Corporation. Located in Pomona, California, this group offers systems engineering expertise in MS, atmospheric monitoring, optical spectrometry and radiometry, GC, and IMS. They provide ground systems and software infrastructure, launch vehicles infrastructure, and satellites, sensors, and electronics products and have sales of approximately $35M.

Over 65 percent of their business is with the government; the commercial sector constitutes the rest of their business market. Some of the commercial instruments that Orbital manufactures are an in-line process analyzer for refinery fluid streams that provides real-time analysis of gasoline, diesel oil, and refinery products; a Methods Validation Platform that provides a NIR analyzer to assure correlation with in-line measurements; a multiple gas analyzer to monitor gaseous flows and effluents for use in steel, fermentation, pharmaceutical and chemical processing, and a sulfur recovery process analyzer to measure liquid amine streams.

The Sensor Systems Division has a 134,000 square foot facility that contains engineering offices and a test lab for the design, development and rapid prototyping of engineering models. The division has production facilities totaling 52,550 square feet, which are fully equipped to support the manufacture and test of hardware, including such capabilities as high reliability assembly, precision assembly, and environmental test labs.

Orbital's Sensor Systems is pursuing the CB detection segment of NBC defense market by leveraging their existing technologies and expertise in the space and commercial sensor products.

### *Technology Development*

Sensor Systems Division technologies for CB warfare agent detection include:

- Mass Spectrometry
- Ion Mobility Spectrometry
- Gas Chromatography
- GC/Flame Photometric Detection
- Surface Acoustic Wave Sensors.

In conjunction with Oak Ridge National Laboratory, Orbital is currently developing the CBMS Block II, for SBCCOM. This system consists of an analyzer unit and a biosampler/concentrator/pyrolyzer unit. The system is based on direct sampling of biological particulates, requiring no reagents and minimizing the logistical burden and operating costs. Designed for use in reconnaissance vehicles and mobile analytical laboratory systems, it offers reductions in weight, size and power consumption over the current CBMS system. Targeted applications for the upgrade are the BIDS, NBCRS FOX vehicle, Lightweight NBC Reconnaissance System, and other biological identification systems.

Orbital also produces the GI-CAD, a chemical agent detection system based on GC and IMS technologies and using Orbital’s proprietary two-dimensional detection algorithms. The system can be used inside a reconnaissance vehicle or dismounted as a stand-alone unit and will be capable of rapidly detecting chemical warfare agents in parts per trillion concentrations. Applications for the system are:

- Field portable detector
- UAV point detector
- Reconnaissance vehicles
- Tech escort missions
- Environmental monitor.

Orbital has developed a Central Atmosphere Monitoring System using MS to continuously monitor life and trace gases aboard U.S. nuclear submarines.

Among the other field instruments offered by Orbital is the chemical detection module - a sorbent trap, (GC/IMS) system integrated into a detector that is being developed for a classified application. Production units are under development.

### *Research International*
### *Woodinville, Washington*

### *Company Overview*

Research International, located in Woodinville, Washington, develops, manufactures and markets sensors and sensing systems. The company was founded in 1990 and has 26 employees. The company specializes in contract R&D in optical CB sensing, micromachining and micro fluidics, and miniature battery technology. Areas of expertise include optics, fluidics, micromachining, electronics, chemistry, biochemistry, plastic molding, and software development.

Research International emphasizes hardware implementation of all R&D efforts and maintains an on-site machine shop plus microfabrication and chemistry/biochemistry laboratories. Their manufacturing department, containing electronics, optics, and mechanical assembly areas, is designed for small to medium production runs.

### *Technology Development*

Research International has developed several systems for use in the detection of CB warfare agents. Their RAPTOR™ Biowarfare Detection system is an automatic fluorometric assay portable (6.3 kg) 4-channel system for monitoring biological agents, toxins, explosives, and chemical contaminants. The self-contained instrument integrates optics, fluidics, electronics, and software into one compact system for laboratory and field assays. The system can detect the presence of minute concentrations of viruses, bacteria, and toxins in liquid samples. The breadbox-sized biosensor runs specific antibody-based assays in a disposable cartridge the size of a credit card. Each cartridge is reusable until a positive response is found and liquid samples ranging from sewage to pureed hamburger can be processed. It performs user-defined, multi-step, assay protocols for monitoring fluorescently-labeled chemical reactions occurring on the surface of each of the system's four disposable optical waveguide sensors. Toxins and bacteria such as ricin and B. anthracis have been detected at levels below 1 ppb.

The Smart Air Sampler System (SASS) is a portable, low-power CB detection device developed for use with a rapid identification system, such as the RAPTOR™, to provide early warning of pathogen-contaminated air in battlefield and urban threat scenarios. This air sampler is capable of concentrating airborne particles by several hundred thousand times into a small amount of water. The SASS 2000 is a multi-stage, wetted-wall cyclone sampler that extracts chemical and particulate-based threat agents from surrounding air and then transfers them to a liquid phase for detection and analysis. The SASS 2000 has been field-tested at government facilities. Company representatives stated that its weight and power consumption are far below other comparable biological warfare collection systems. They are further reduced in a ram-air driven model in unmanned air vehicles.

The Analyte 2000 is a 4-channel, single wavelength biological detection fluorometer usingevanescent-wave fluoroimmunoassays that was developed in conjunction with the Naval Research Laboratory for biomolecule detection. Company representatives stated that this low-power, microprocessor-controlled instrument provides parts-per-billion sensitivities to biochemical species, such as proteins, viruses, bacteria, and spores, by monitoring antibody/antigen reactions on tapered glass or plastic waveguides. The instrument is controlled from a remote computer leading to a system size of only 20 cm L x 8.5 cm H x 11.2 cm W. The Analyte 2000, coupled with a separate fluidics box, has been flown and operated in small, unmanned air vehicles using a remote RF link to a ground-based portable computer. The Analyte Adapter is a small opto-electronics module that serves as an interface between the Analyte 2000 and the inexpensive, injection-molded polystyrene waveguides developed for use with the RAPTOR fluoroimmunoassay system.

The Flow Assay Sensing and Testing (FAST) system, developed under contract to the Naval Research Laboratory, is a rapid portable chemical warfare detection system for performing flow immunoassays with detection limits to 1 part-per-billion. The FAST 2000 was initially developed for detection of RDX and TNT in water, and assays are currently being developed for chemical warfare agent detection applications.

The FAST 6000 can be configured either as a single channel, single analyte instrument or as a six channel, six analyte instrument, capable of performing six simultaneous assays for six different analytes on the same sample. The system is self-contained, needing only a bottle of buffer and a waste bottle for field operation. In operation, this small-molecule assay system uses a fluorophore-labeled analyte bound to analyte-specific antibody that is immobilized on a permeable membrane. When a sample containing analyte passes through the membrane, an exchange reaction occurs, displacing labeled analyte that is measured downstream. A typical assay requires two minutes to run and up to 50 assays can usually be performed before the disposable membrane coupon has to be recharged.

During the 2000 World Trade Organization meeting in Seattle, Washington, the Seattle Fire Department evaluated the RAPTOR™ and the SASS 2000. In the week prior to the World Trade Organization meeting, four Hazardous Materials Response Teams were trained on the equipment and provided with a supply of assay cartridges specific for anthrax.

### *Sensors for Medicine and Science, Inc.*
### *Germantown, Maryland*

### *Company Overview*

Sensors for Medicine and Science, Inc.(SMSI), located in Germantown, Maryland, is a venture capital company founded on January 15, 1997. SMSI has approximately 20 employees. The company's focus is on commercializing their patented sensing technology. This patented technology permits sensitive detection and measurement of molecules. The company also has exclusive rights to additional related technology.

SMSI has developed a solid-state optical-sensor configuration that can serve as a development platform for a host of chemical and biochemical sensors in either gaseous or liquid environments. The device has been implemented for oxygen sensing via fluorescence quenching and offers potential advantages over existing electrochemical and more recent fiber-optic methods. According to company representatives, this platform technology features enhanced energy efficiency; high-sensitivity; low-power consumption; ease of miniaturization; low-cost, high-volume manufacturability using standard methods; very fast response/recovery profiles; and high reliability.

The company's platform enables sensing approaches based on fluorescence, absorbance and refractive index. The Company's preferred embodiment involves fluorescence-based sensing. SMSI representatives believe that this technology can be used for applications in the medical, industrial and environmental industries. Currently, they have not completed any commercial sales, but rather are still conducting R&D of this technology. The company has a commercial partner for certain products employing SMSI's oxygen sensor.

Company representatives envision that their first commercial sales will be sales of their oxygen sensor for respiratory monitoring to their marketing partner sometime in 2001. Their next goal is commercialization of an implantable glucose sensor, but that is longer term. The glucose sensor is undergoing pre-clinical studies and animal studies; the company will then proceed with human clinical trials and the Food and Drug Administration approval process. Following this, the company plans to focus on adaptation of this same sensing platform for other analytes.

Once key features of the sensor platform – the electronics and optics - are finalized, then it becomes strictly a matter of substituting sensing chemistries. Researchers could take this same platform and adapt it to a new sensing chemistry, thus transforming the system into a sensor for a different analyte.

Company researchers envision that the technology is potentially applicable to a wide range of molecules to be measured (analytes). Medically relevant analytes include oxygen, carbon dioxide, pH, glucose, lactate and anesthetic gases. They also perceive market opportunities for sensing of most of these same analytes outside the medical field. One example cited was oxygen sensors, that may have non-medical applications such as food packaging and formulation, fermentation, semiconductor plating, corrosion control and pollution monitoring.

The core, patented technology was developed by SMSI. The company is also collaborating with the Johns Hopkins University APL on this technology, which has utility in both commercial and defense applications. Among other activities, APL scientists are conducting performance and reliability tests of the sensor, including reliability tests under varying environmental conditions.

Company representatives noted that their commercial development strategy involves:

- Sales of sensors to high value, end-user markets
- Option and licensing fees, research funding, milestone payments, and royalties from corporate partners and licensees who are granted licenses to the Company's technology in defined fields of use
- Manufacture of sensor elements for sale to licensees.

SMSI has raised a total of $15M of financing in two series of convertible preferred stock issuances. Five firms participated in the financing, including New Enterprise Associates, which provided the company's seed funding. Other investors include HealthCare Ventures, Abingworth Management Limited, Rho Management, and Anthem Capital L.P.

### *Technology Development*

SMSI researchers stated that, today, scientists use fiber optics and lasers in bench-top spectroscopy configurations that can cost tens of thousands of dollars. The oxygen sensor prototypes that SMSI has developed, on the other hand, uses a tiny Light Emitting Diode (LED) as the light source, and an ordinary photodiode - like the ones in solar powered calculators - to measure the light. The essence of the SMSI invention was to place the excitation source in the sensor element. Specifically, they've embedded the tiny, low cost LED in a matrix containing the fluorescent indicator molecules (fluorochrome). This allows both cost savings and a reduction in weight and size, since both are widely available, relatively inexpensive, and are small.

SMSI has developed this working oxygen sensor based on surface mount printed circuit board technology. The final stage of the Company's plan is to advance the platform to a monolithic sensor on a chip. Company researchers believe that this approach will significantly reduce both the size and, in volume, the cost of the sensor. The final stage version is expected to have dimensions in the hundreds of microns. Adaptation of the indicator chemistry to permit sensing of additional analytes may follow a somewhat independent path. This effort will be prioritized by the market opportunities of interest to SMSI and its partners or licensees.

SMSI is also developing an implantable glucose monitoring chip that would be inserted under the skin. The chip will allow diabetics to easily monitor the level of glucose in their blood. Diabetics currently use a skin prick and a hand-held blood test, and then medicate themselves with insulin depending on the result. SMSI spoke-persons noted that the current process's requirement to draw blood frequently means that most diabetics don't test themselves as often as they should. Although they may get away with this in the short term, in later life those who monitored infrequently typically suffer from blindness, loss of circulation, and other complications. SMSI's proposed chip would dramatically improve this step of the process.

A LED starts off the detection process. The light that it produces hits a fluorescent chemical: one that absorbs incoming light and re-emits it at a longer wavelength. The longer wavelength of light is then detected, and the result is sent to a small remote reader in the form of a wristwatch or pager-like device.

Glucose is detected because the sugar changes the amount of light that the fluorescent chemical re-emits. The emitted light changes in proportion to changes in ambient glucose levels. SMSI has developed working fluorescent indicator chemistries for glucose, and continues to pursue potential alternative chemistries. Although SMSI continues to develop its glucose indicator chemistries, the key design innovation of the SMSI chip has been fully worked out. The LED sits in a sea of the fluorescent molecules. In most detectors, the light source is far away from the fluorescent molecules, and the inefficiencies that come with that mean more power and larger devices. One prototype SMSI chip uses a 22µW LED, almost 40 times less powerful than the tiny power-on indicators on a computer keyboard. The low power requirements mean that energy can be supplied from the outside. The fluorescent detection itself does not consume any chemicals or proteins, so the device is self-sustaining.

### *University of Alabama*
### *Birmingham, Alabama*

### *Overview*

The University of Alabama (UAB) is working on developing reagents for use in biological detectors. Their research is focused on a ligand that binds tightly and specifically to a particular bio-agent. They envision that this ligand could be tagged with a detectable marker, and the addition of this ligand-tag conjugate to unknown samples would reveal the presence of the cognate bio-agent. The scientists also believe that such a ligand could also be incorporated into other detection devices as a means of capturing and concentrating bio-agents prior to direct analysis for strain-specific characteristics (e.g., unique DNA sequences and surface maromolecules).

The UAB Automated DNA Sequencing Core Facility currently has two ABI PRISM 377s, one ABI Prism 373 and one ABI Prism 310 instruments. The ABI 377 is designed for high throughput. It can be used for DNA sequencing or for fragment sizing and quantitative analysis, but it can only be used for one of these two types of analysis at a time. The instrument is also capable of performing automated analysis of dye-labelled DNA/RNA produced by PCR; microsatellite genotyping of DNA; gene expression studies; and analyses of gene mutations.

### *Technology Development*

Two major research thrusts at the University laboratory involve alternative molecular recognition technologies based on DNA based recognition. The first project focuses on the use of reiterative transcription in gene regulation in Escherichia coli. Reiterative transcription is the repetitive addition of a nucleotide to the 3' end of a nascent transcript due to slippage between the transcript and DNA template. Recent studies in the University lab have shown that reiterative transcription during initiation plays a central role in pyrimidine -mediated regulation of the pyrBI, carAB, codBA, and upp operons, which are involved in either pyrimidine biosynthesis or salvage. In each case, reiterative transcription produces transcripts that are not extended past the initially transcribed region of the promoter, and thus reduce operon expression. However, the mechanism that controls the extent of reiterative transcription at the pyrBI and carAB promoters is fundamentally different than the mechanism operating at the codBA and upp promoters. Researchers at the University are presently studying the role of reiterative transcription in the regulation of other operons, most of which are not involved in pyrimidine metabolism. They anticipate that these studies are likely to elucidate new mechanisms of gene regulation. In addition, studies are in progress to define the mechanism of reiterative transcription and the factors that modulate the extent of this reaction.

The second major project is to identify ligands that can be used for the capture and identification of bio-agents, particularly spore-forming bacteria. A central component of this work is the use of phage-displayed peptide libraries to identify short peptides that bind tightly and species-specifically to target spores. The researchers are also characterizing the spore receptors to which the peptide ligands bind, as well as any spore coat proteins that use the peptide-ligand sequence to bind the spore surface, perhaps during spore formation or maturation.

### *University of Texas*
### *Austin, Texas*

#### *Overview*

The Institute for Advanced Technology (IAT), at The University of Texas at Austin was founded in 1990. IAT is an autonomous research unit under the Office of the Vice President for Research. The Institute supports the U.S. Army with basic and applied research in electrodynamics, hypervelocity physics, pulsed power, and education in related critical technologies. The IAT has a large antibody and biosensor development program aimed at developing CB warfare sensors. The researchers are striving to use high-throughput and automated evolutionary engineering techniques to create antibodies, aptamers and aptazymes against all known BW targets. They intend to use biosensors with overlapping specificities to try and detect previously unknown agents. The University's scientists are mounting the evolved biosensors on an 'electronic tongue' platform for optical detection, but are also interfacing with military agencies and suppliers to include the University's biosensors in their devices.

The University receives funding from a number of government sources, including DTRA, DARPA, Office of Naval Research, and Army Research Organization. They are collaborating on CB warfare sensor R&D initiatives with researchers at UT Southwestern, UTMB in Galveston, UT San Antonio, Texas A&M, Texas Tech, and Rice.

#### *Technology Development*

The technologies that the University's scientists are researching in the receptor/sensor development arena are:

- High-throughput antibody identification
- Conversion of antibodies to biosensors for 'reagentless' formats
- High-throughput aptamer identification
- Conversion of aptamers to biosensors for 'reagentless' formats
- Development of 'aptazymes,' extremely novel reagents for signal transduction
- Development of combinatorial chemical libraries of 'smart dyes' for sensing
- Design of 'smart dye' sensors
- Enzyme-based sensors for CB warfare agents

All of the above efforts are being carried out with a suite of CBW agents that the scientists keep in a P3 facility. They are involved in discussions with a number of outside collaborators that could potentially adapt these sensors to their particular platforms. In addition, the researchers are mounting these sensors on several platforms being developed internally.

In regards to platform development, the University is involved in developing an 'electronic tongue' (microbeads in microwells) and patterned surfaces. The electronic tongue is being commercialized by Labnetics, a company in Texas.

Finally, in addition to exploring sensitive optical detection, the researchers are involved in the development of a new bioelectronics community that should provide direct interfaces between biomolecules and electronic devices. The University team of scientists has recently identified peptides that can specifically interact with semiconductor surfaces, and is using these peptides to both direct the construction of novel electronic materials and to directly interface peptide sensors with semiconductor devices.

### *University of Virginia*
### *Aerogel Research Laboratory*
### *Charlottesville, Virginia*

#### *Laboratory Overview*

The University of Virginia Aerogel Research Laboratory was established in 1996 with the mission to investigate both the fundamental properties and the cutting edge applications of aerogels. University researchers explained that aerogels are highly porous solids made out of materials such as silica, alumina, or zirconia. Silica aerogel, one of the most common forms of aerogel, consists of mostly air with the remainder being a wispy matrix of silica. Silica aerogel is the lowest density solid material ever fabricated and it has the lowest thermal conductivity and the lowest dielectric constant of any material known.

The lab is currently investigating:

- microscale energy diffusion in these fractal materials;
- development of an aerogel based collection media for detection of BW agents;
- development of a thin film aerogel polymer material for use as a low dielectric constant substrate for the microelectronics industry; and
- development of a non-contact technique for measuring the index of refraction and thickness of porous thin film materials.

The laboratory is equipped with complete facilities for producing aerogel in thin film, microsphere, and bulk (monolithic) form.

#### *Technology Development*

The University is under contract to Veridian-PSR to develop smart aerogel coatings for 3-D microchip-based technology that will enhance bio detection. Initiated in June, 1999, the researchers are working to develop gel-pad coatings with a high density of oligonucleotide sites that both recognize the target agent and trigger an optical response. Their focus is on developing a new sol-gel approach to the formation of custom-designed micro-, meso- or macroporous receptor gel-pad coatings.

University researchers noted that typical sol-gel polymers possess a complex, adjustable pore structure, high internal surface area, high porosity, and adjustable surface chemistry which together render a highly efficient, scaffold for integration with a microchip platform. The high surface area matrix would serve as a 3-dimensional scaffold for attachment of rRNA recognition elements, resulting in increased output signal and absorption capacity. By using modified inorganic sol-gel pads arranged in microarrays, they hope to develop a matrix that is printable, storable, requires no successive washing and depositions, has a quantifiable probe activity and displays decreased non-specific target adsorption

On a separate contract with Pacific Sierra Research, they are working to develop a multi-functional material, called smart aerogel, into a breadboard prototype biosensor. Aerogel properties of complex pore structure, high internal surface area and hygroscopicity are being exploited to synthesize a smart collection medium that is internally coated with bioaffinity compounds with high specific binding potential to unique pathogens. The researchers noted that this results in an isolation of the pathogen by size and type that allows area-limited signal transduction to achieve simultaneous detection and identification. Signal transduction is being investigated for a number of means that include optical, acoustical, electrical, and olfactory assay. The researchers believe the resulting sensor concept has the potential to meet rigorous functional requirements of the next generation biosensor with the added advantage of making it extremely lightweight and small for deployment on micro-sized airborne vehicles.