Report on the Integrated Chemical and Biological Defense Research, Development and Acquisition Plan *for the* Departments of Defense and Energy

BIO POINT DETECTION

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March 2001

20040517 021

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ABBREVIATIONS AND ACRONYMS

ACEs	Areas for Capability Enhancement
ACPLA	Agent-Containing Particle per Liter of Air
ACTD	Advanced Concept Technology Demonstration
AMB	Advanced Multifunction Biochip
APDS	Autonomous Pathogen Detector System
ATD	Advanced Technology Demonstrations
BAM	Business Area Manager
BASIS	Biological Aerosol Sentry and Information System
BSPS	Biological Sample Preparation System
BW	Biological Warfare
BWD	Biological Warfare Defense program
CBD	Chemical and Biological Defense
CBDP	Chemical and Biological Defense Program
CBNP	Chemical and Biological National Security Program
CDC	Center for Disease Control
CPRC	Counterproliferation Program Review Committee
CRP	Critical Reagents Program
DARPA	Defense Advanced Research Projects Agency
DDAP	Domestic Demonstration and Application Program
DI&W	Detection, Identification and Warning
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
DOE	Department of Energy
ECBC	Edgewood Chemical Biological Center
ELISA	Enzyme Linked Immunosorbent Assay
EMD	Engineering and Manufacturing Development
ESI	Electrospray Ionization
FDA	Force Differentiation Assay
GFE	Government Furnished Equipment
HPLC	High Performance Liquid Chromatography
JCBAWM	Joint Chemical Biological Agent Warfare Monitor program
JFOCs	Joint Future Operational Capabilities
JFT	Joint Field Trials
JHU-APL	Johns Hopkins University Applied Physics Laboratory
JMCBD	Joint Modular Chemical and Biological Detector
JPBDS	Joint Point Biological Detection System
LANL	Los Alamos National Laboratory
LLNL	Lawrence Livermore National Laboratory
MAGIChip	Micro Array of Gel Immobilized Compounds on a Chip
MALDI	Matrix-Assisted Laser Desorption Ionization
MASINT	Measurement and Signature Intelligence
NBC	Nuclear, Biological and Chemical
ORNL	Oak Ridge National Laboratory
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PM	Program Manager
POM	Program Objective Memorandum

PROTECT	Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism
PY-GC/IMS	Pyrolysis-Gas Chromatography/Ion Mobility Spectrometry
R&D	Research and Development
RDT&E	Research, Development, Test and Evaluation
RDA	Research, Development and Acquisition
S&T	Science and Technology
SDA	Strand Displacement Amplification
SESI	Science and Engineering Services Incorporated
SNL	Sandia National Laboratory
TOF	Time of Flight
TSWG	Technical Support Working Group
UCP	Up-Converting Phosphor
UCPFCM	Up-Converting Phosphor Flow Cytometer
UCPHHA	Up-Converting Phosphor Handheld Assay
USAMRIID	U.S. Army Medical Research Institute of Infectious Diseases

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Integrated Chemical and Biological Defense Research, Development and Acquisition Plan for the Departments of Defense and Energy: Biological Point Detection

Introduction

This report serves a dual purpose. First, it fulfills Counterproliferation Program Review Committee (CPRC) and Congressional coordination and reporting requirements[1] for the Department of Defense (DoD) and the Department of Energy (DOE) in the area of chemical and biological defense (CBD) research, development and acquisition (RDA). The first CBD RDA report published in April 2000[2] explained the rationale for and genesis of interagency coordination via the CPRC-chartered CBD RDA Focus Group and the roles and responsibilities of DoD and DOE and other agencies. This report focuses in detail on a specific technology area—biological point detection.

It is this narrower and more detailed scope that allows the report to serve its second and equally important purpose. This report is a "living" document intended to facilitate coordination and cooperation between DOE and DoD at both the high level of national policy and planning and at the working level in the area of biological point detection.

The report achieves these objectives through a detailed "roadmap" that depicts R&D programs within the DoD Chemical and Biological Defense Program (CBDP), Defense Advanced Research Projects Agency (DARPA) and the DOE Chemical and Biological National Security Program (CBNP) as well as plans for testing and transitioning technologies into the acquisition process through FY10. Program data for this extended timeframe comes from existing planning documents in many cases; however, it should be noted that appearance within the roadmap does not imply funding commitments over that period. Rather, the integration of these efforts into a single planning document represents a significant step toward a more formal, unified, long-term investment strategy.

This roadmap allows agency leaders to have visibility across current and planned RDA efforts to avoid duplication of effort and to identify possible synergies and relevant research performed by their partner agencies. The sponsors of this report, the CBD RDA Focus Group,[3] hope that it will provide useful information to Principal Investigators (PIs) and Program Managers (PMs) as well. The report is also intended to inform and enhance interaction among R&D scientists. In addition to the initial biological point detection roadmap, the report defines a general annual process to be used by the Focus Group for developing and then updating similar roadmaps for other technology areas. The biological point detection roadmap is thus a model to be reviewed, modified, and then applied across all CBD RDA technology areas.

The biological point detection roadmap, taken together with the other technology roadmaps, constitutes a single Integrated Plan.

Methodology

The goal of the CBD RDA Focus Group was to produce an integration template applicable across technology areas to facilitate interagency coordination and cooperation. The template has two essential elements.

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[1] See Appendix C for the congressional language establishing these requirements.

[2] Integrated Chemical and Biological Defense Research, Development and Acquisition Plan for the Departments of Defense and Energy, Counterproliferation Review Committee, April 2000.

[3] The Focus Group includes decision-makers and area experts from CBDP, DARPA and CBNP.

- A format for an integrated technology area roadmap
- A repeatable process by which the roadmap is first developed and then annually reviewed and updated

The methodology for developing these elements was to create a "pilot" template and process for a selected part of the Integrated Plan—a key part of a single technology area. The resulting pilot template and process will be repeatedly tested and improved as it is applied across the remaining R&D and transition activities in all technology areas to culminate in a comprehensive Integrated Plan. This initial roadmap and plan can be maintained and updated annually and expanded to additional technology areas when desired. In order to facilitate successful development of a general template, the methodology included two key means to bound the problem of template development.

Limited Participation in Roadmap Development. In order to simplify initial template development, the Focus Group included in this roadmap only those activities funded by its own membership—CBDP and DARPA from DoD and DOE CBNP. A future goal is to include broader representation from the user/developer community in development of integrated plans.

Selection of a Technology Area Focus. Also to bound the effort, the Focus Group selected a single technology sub-area, biological point detection, from the many CB defense technologies presently under development.[4] Biological point detection is only one element of the detection technology area (see figure below), but biodetection is a top national priority for both battlefield and domestic response users and has received significant funding emphasis as a result.

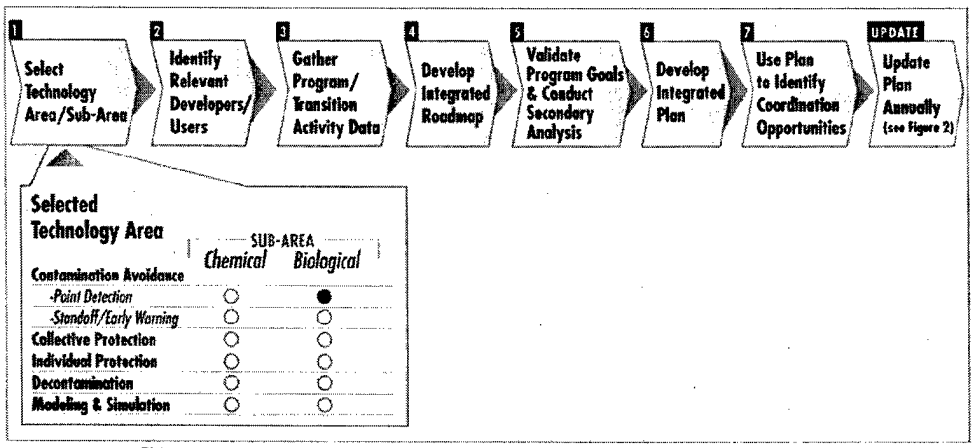


Figure 1. Technology Roadmap Development Process

There are many opportunities, but also some important constraints, in moving the developmental technologies to full maturity and into the acquisition phase. Coordination and cooperation are therefore particularly important in biological point detection to facilitate maximum leveraging of resources and timely technology development and transition to the acquisition process.

Selection of a technology area and identification of stakeholders (developers and users) are the first steps in the roadmap development process. In this initial instance of process definition, they were done in reverse order from the model for two reasons.

[4] Other key technology areas include collective protection, individual protection, decontamination, the other sub-areas of contamination avoidance, and modeling and simulation. As with detection, each of these technology areas comprises several sub-areas. One of the next steps for the Focus Group will be to develop a technology taxonomy and prioritize technology areas for development of additional integrated plans.

- Focus Group members were tasked to build the whole model. They chose to limit the task to a specific technology area initially.
- Simplifying the development of the model roadmap necessitated limiting user/developer participation. Thus, the Focus Group selected a technology area that was within its combined expertise.

The next step in the Focus Group's approach was to determine what specific data are relevant to increased interagency RDA transparency. The following data were gathered in the development of the model roadmap.

- Timelines for anticipated acquisitions (*e.g.*, Engineering and Manufacturing Development programs (EMD) such as the Joint Point Biological Detection System (JPBDS) and the Joint Modular Chemical and Biological Detector (JMCBD[5]))
- Top-level guidance (Joint Future Operational Capabilities (JFOCs), Areas for Capability Enhancements (ACEs))
- Testing and demonstration activities and key events (*e.g.*, Advanced Technology Demonstrations/Advanced Concept Technology Demonstrations (ATDs/ACTDs), Joint Field Trails (JFTs) and Domestic Demonstration and Application Programs (DDAPs))
- Technology area R&D programs—timelines and key milestones, including participation in testing and demonstration activities.

By showing R&D program timelines, transition timelines, and testing activities in a single graphic, the roadmap provides a panoramic look ahead for a given technology area. The above data must be gathered from each agency's existing planning documents, PMs and PIs in order to complete the roadmap.

As Focus Group members developed the format for the technology area roadmaps, they also began to define an annual process for integration and updating them. Figure 2 depicts the annual process concept; note that there are no set timelines, with the exception of the annual meeting of the Roadmap Committee. This is because, although the process is applicable to all technology area roadmaps, it will involve interaction with some different sets of developers and users for each, so the timing of annual cycles may differ slightly. Ideally, all roadmap updates should be completed in time to influence development of investment strategies.

[5] See Appendix A for a description of these programs.

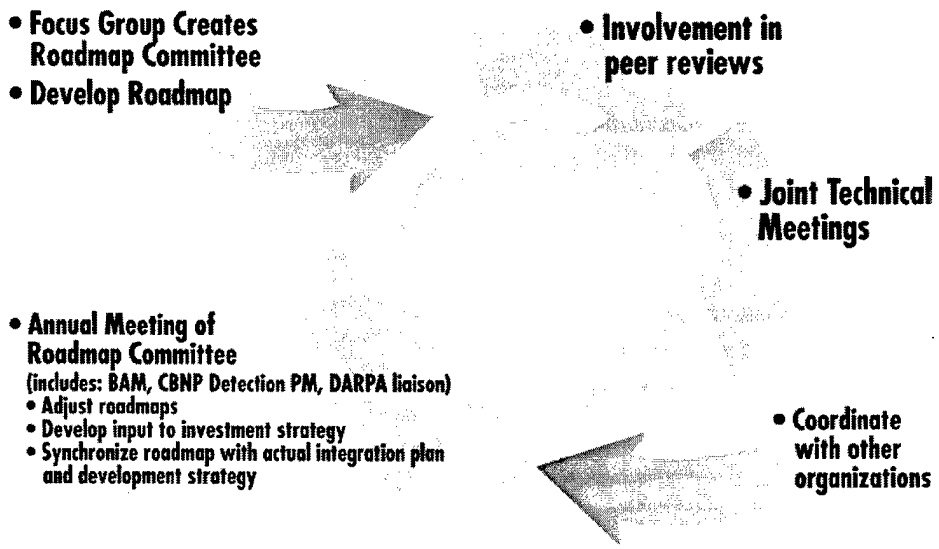


Figure 2. Annual Integration Process and Roadmap Update Cycle

The process steps depicted in Figure 2 and briefly described below apply to all technology area roadmaps, but examples and details are based on the biological point detection roadmapping process. It should be noted that the process is still under development and has yet to be tested and modified.

The process includes the following steps.

- Establishment of the Roadmap Committees, which are technology area sub-committees of the Focus Group; this occurs only once, during overall process development. The Focus Group identifies Roadmap Committee members for each technology area, and has done so for biological point detection. Roadmap committees include, at a minimum, the DoD Joint Service Business Area Manager (BAM), the DOE CB National Security Program PM, and a DARPA liaison.
- Establishment of the initial technology area roadmaps, once for each technology area, by the designated Roadmap Committees. These initial roadmaps are then reviewed and adjusted annually.
- Cross-organizational involvement in peer reviews, to facilitate identification of any potential duplication of effort or opportunities to cooperate and leverage synergies across programs and demonstrations. This is an ongoing activity, but should acquire new emphasis as the roadmapping process proceeds. This cross-organizational involvement is required to deconflict planning and develop shared expectations across the technology area community.
- Regular joint detection meetings at the PI level to ensure continuous progress on deconfliction and integration. These meetings must occur at least annually for each technology area Roadmap Committee to review and update the roadmaps in time for the annual spring meeting of the Focus Group. Each Committee has the latitude to select an opportune technical conference around which to schedule an update meeting. A cross-organizational meeting of PIs in the area of mass spectrometry at the Joint Service Conference on Point Detection held in Williamsburg, Virginia in October 2000 is an example of this type of meeting. The improved cooperation that has been developed in this area is discussed in more detail in Appendix B.
- Special focus technology meetings that bring in key members of the user-developer community, when deemed appropriate by the detection PIs, the technology area Roadmap Committee, or the

Focus Group. Such meetings may occur if a developing technology is under consideration for inclusion in a transition testing or demonstration activity, for example.

- Coordination with other organizations, including the intelligence community (IC) overall and specific organizations, such as the Measurement and Signature Intelligence (MASINT) organization for biological point detection, Technical Support Working Group (TSWG), as well as other CPRC member organizations.
- The annual roadmap development, review, and update process will culminate in a spring meeting of the Focus Group to review progress on all technology area-specific CBD RDA plans; adjust the roadmaps as needed; and develop investment strategies. It is critical that this meeting take place in the spring, in time to affect the POM update cycle.

The Roadmap Template

The first product defined in the above methodology is a template for the Technology Area Roadmaps. The intent of the Roadmap template is to provide a tool for depicting DoD and DOE R&D programs and the means and timing of their integration into testing, demonstration, and acquisition activities in order to facilitate cross-organizational awareness and cooperation. Such cooperation will assist in eliminating unnecessary duplication of DoD/DOE R&D efforts as well as provide a means for productive interagency leveraging. The draft Biological Point Detection Roadmap—the model to be applied to other technology areas—has already stimulated improved cooperation across Mass Spectroscopy R&D programs (see the Findings section of this report).

Figure 3 shows the general roadmap template, which was used to develop the draft Biological Point Detection Roadmap. The template is separated into two main sections. The top section consists of Acquisition/Transition Activities, whereas the lower section comprises Sensor/System R&D Programs in a given technology area. Funding and executing entities responsible for these activities and programs are listed in the column on the far left. The Acquisition and Transition Activities listed are exercises/events that provide technology insertion points for Sensor/System program deliverables. Acquisition/Transition Activities include CBDP EMD programs, DOE DDAPs, DoD ATD/ACTDs, DoD/DOE Test/Validation Programs, and Guidance documents.

The Roadmap is color coded for clarity. In this example, CBNP and DARPA activity/program titles on the left are shaded in orange and light blue, respectively. CBDP activity/program titles are not shaded. The timeline bars associated with each Acquisition/ Transition Activity are also uniquely colored. Black and white diamonds are placed within these timeline bars to denote a major test or demonstration will take place in a specific activity. Black diamonds designate "hard" milestones—those at which a firmly scheduled activity or event occurs. White diamonds represent "soft" milestones—timing goals rather than firm events. Sensor/System R&D program involvement in an Acquisition/Transition activity is shown on the program timeline bars. A block in color denoting the Acquisition/Transition activity is inserted to depict the specific test/demonstration and time period that an R&D deliverable will be tested or demonstrated.

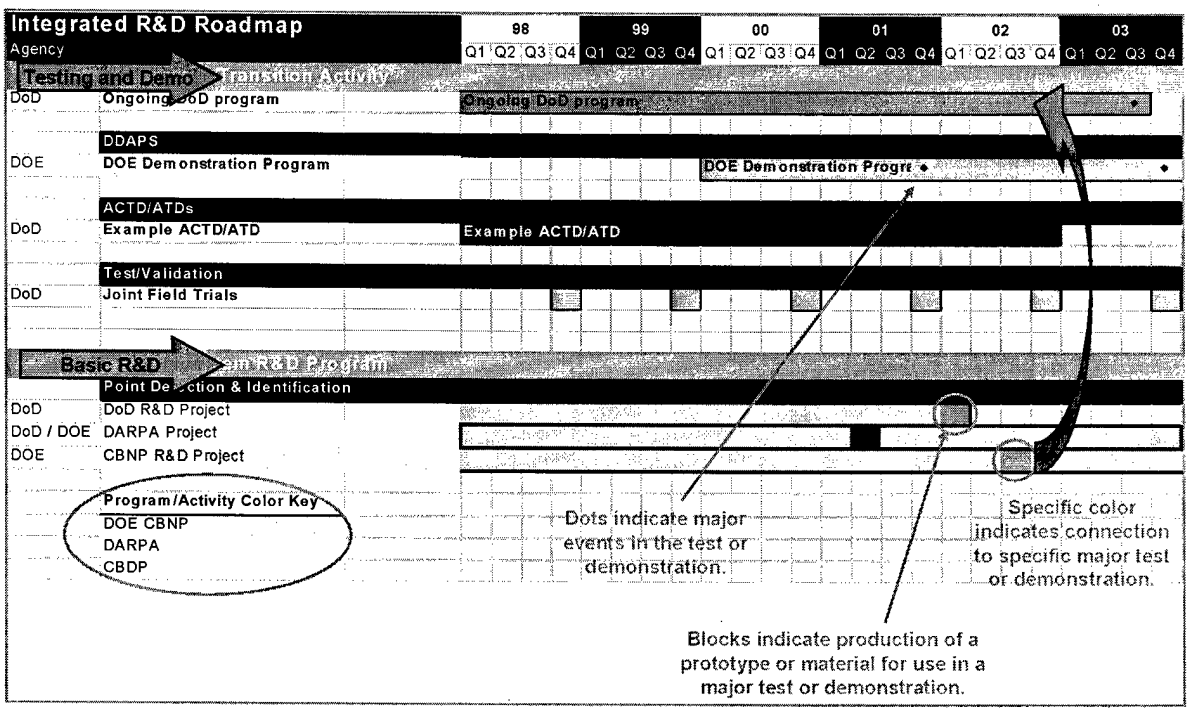


Figure 3. The General Roadmap Template

The Biological Point Detection Roadmap

The first iteration of the Biological Point Detection Roadmap is shown in Figure 4 on the following page; details of the programs included on the roadmap are provided in Appendix A. The Roadmap covers relevant Acquisition/Transition activities and R&D programs from FY 99 through FY 10. The next biological point detection roadmap will include activities sponsored by other organizations in addition to CBDP, DARPA and CBNP.

Acquisition/Transition Activities Involving Biodetection Technologies

The transition and acquisition activities to which DoD and DOE biological point detection R&D programs make significant contributions include three programs depicted during their EMD acquisition phase (JPBDS, JCBAWM, and JMCBD), three DOE Domestic Demonstration and Application Programs (DDAPs) (PROTECT, BASIS, and Bioforensics), and several types of testing and validation programs. The DoD activities, as well as the supporting R&D programs, contribute to requirements developed in two key pieces of guidance.

- The Joint Future Operational Capabilities (JFOCs) list developed by the Joint Services.
- The prioritized CPRC Areas for Capability Enhancement (ACEs).

Activities and programs in the Biological Point Detection Roadmap support the development of warfighting capabilities for ACE 2: Detection, Identification, Characterization, and Warning of CBW agents.

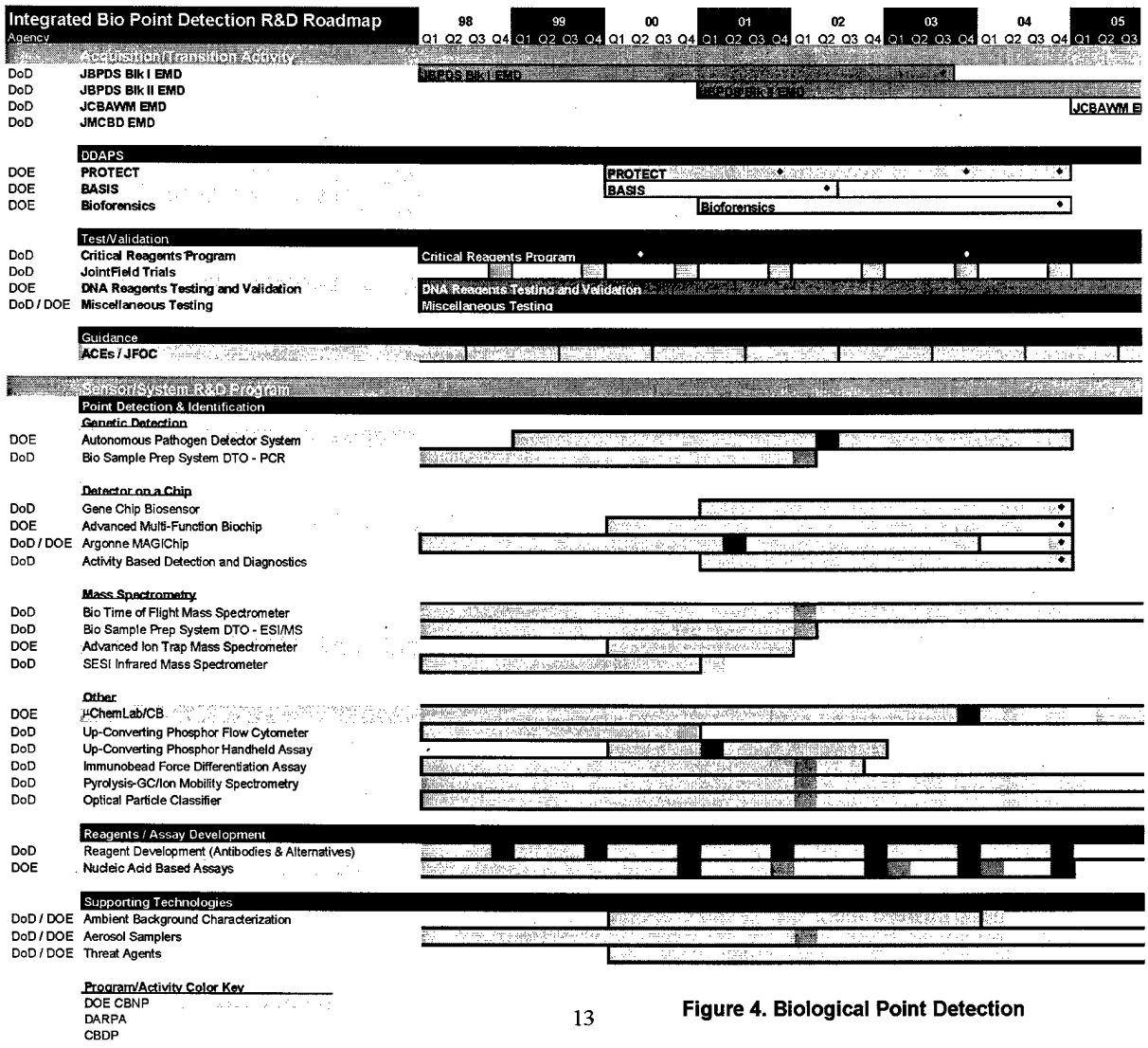


Figure 4. Biological Point Detection

Biological Detection and Identification: Current Programs and Projects

Sensor/System R&D programs include Biological Point Detection and Identification, Reagents/Assay Development, and Supporting Technologies. Biological Point Detection and Identification Programs are further subdivided into major activity areas: Genetic Detection, Detector on a Chip, Mass Spectrometry, and other programs that have not yet been categorized. In developing the roadmap, the Focus Group identified several "like" R&D programs that have been grouped together. While three of these groups are based on common technology platforms, their approaches explore different ways of applying the underlying technology. Table 1 provides a summary overview of the technology groupings and shared technology platforms. The table also includes supporting technologies that will contribute to the other more mature groups once they are better defined. A detailed description of these activities and programs can be found in Appendix A.

Table 1. Sensor/System R&D Technology Groupings

Technology Group	Programs	Shared Technology Platform
Detection and Identification		
Genetic Detection	• APDS • BSPS-PCR	PCR for genetic detection of bacterial and viral agents
Detector on a Chip	• Gene Chip Biosensor • Advanced Multi-function Biochip • Argonne MAGIChip • Activity Based Detection and Diagnostics	Microchip platform for detection
Mass Spectrometry	• Bio-ToF MS • BSPS-ESI/MS • Advance Ion Trap MS • SESI IR MS	Mass spectroscopy methodologies for sample collection
Other	• µChemLab/CB • UCPFCM, UCPHHA • Immunobead Force Differentiation Assay • Pyrolysis-GC/Ion Mobility Spectrometry • Optical Particle Classifier	N/A—each platform is unique
Reagent/Assay Development		
	• Reagent Development with Antibodies & Alternatives • CBDP Nucleic Based Assays • CBNP Nucleic Based Assays	Goal of programs is shared, but the nucleic acid-based program differs from the antibody programs
Supporting Technologies		
	• Ambient Background Characterization • Aerosol Sampler Development • Threat Agent Characterization	Immature technologies not yet fully defined; will eventually contribute to the biological point detection technologies listed above

Findings

Redundancy Analysis

An important objective of RDA integration between DoD and DOE is to identify and eliminate any unproductive and costly redundancy across programs that may be oriented to different users—battlefield users for DoD and civilian responders for DOE—but which support similar operational capability objectives. Redundancy may also develop within an agency.

However, it is important to understand that, due to the complexity of molecular biological research, a certain amount of complementary research involving exploration of various similar technologies to achieve the same capability objective can increase the probability of success. Complementary research along several similar avenues helps to identify and select the best options more expeditiously than if technology options were explored in linear fashion. It also facilitates synergistic learning, especially if coordination and information sharing are encouraged and rewarded. Truly redundant research, on the other hand, involves a complete duplication of effort that creates not only an unnecessary funding burden but also a loss of valuable research time. By contrast, complementary research along several paths allows for the integration of portions of separate efforts at appropriate stages of development, thus resulting in more efficient development of a more robust end product. Increased probability of timely success is a significant factor to consider given the vast number of biological threats that exist and the fact that the United States does not yet have an optimized biological detection system for military or civilian use. Premature integration and/or down-select decisions would have a negative impact on the development of such systems due to increased risk and a reduction in manpower to continue research and development.

This integration effort has already allowed DOE and DoD R&D programs to promote the type of complementary, coordinated research described above while avoiding true redundancy not only between departments but also across the two agencies. Complementarity and transparency encourage competitive innovation and allow for trial and error. This research and development approach acknowledges that there can be a significant number of novel avenues within the same general technology (e.g., Mass Spectroscopy, PCR). Although it is very early in the process, the DoD/DOE integration effort has resulted in an expansion of cooperation based on complementary research within the area of mass spectrometry.

Mass Spectrometry

The most notable area of cross-organizational cooperation catalyzed by the integration effort is in mass spectroscopy, discussed in Appendix B. This is in no small part due to the fact that initial integration efforts were emphasized with mass spectroscopy because these programs were both more mature and better funded than other technology group programs. As a result of integration efforts early on, both DARPA programs (Bio-ToF and SESI), CBDP BSPS and CBNP Advanced Ion Trap programs were all designed to be novel in defined areas including sample ionization and spectra processing. Consequently, an assessment of these inter- and intra-agency collaborative and leveraging efforts revealed that this detection platform has not demonstrated any duplicative efforts to date.

Future Assessments

There are several other R&D programs that will soon receive the same scrutiny as the Mass Spectrometry technologies. The Reagent/Assay Development R&D group of programs is one that must be considered carefully. Nucleic acid and antibody-based technologies are of utmost importance since they are the foundation of existing and next generation identification platforms. The working combination of these two technologies in a single detection system creates a powerful method of

biological agent identification. PCR probe/primer systems as well as nucleic acid hybridization protocols against a variety of pathogens are currently under development. Similarly, new and improved methodologies for antibody development are underway. It will take numerous inter- and intra-departmental collaborative efforts to ensure that only the most sensitive and specific nucleic acid and antibody reagents are developed. When considering the significant number of biological agents together with their genomic and immunological complexity, optimal reagents can only be developed through competitive innovation and trial and error. This will require a significant amount of manpower and hence care must be taken not to down-select prematurely in these programs.

Cooperative Planning

The integration effort has also had an early influence on sensor system participation in planned acquisition/transition testing. Clearly, the integrated biological point detection roadmap seen above demonstrates a reasonably integrated effort. The current roadmap represents substantial progress in cooperative planning over the initial roadmap that was generated in the early stages of the integration effort. For example, comparison of the old and new versions shows a significant increase in the number of interagency integration opportunities that have been identified and will now be exploited for sensor system R&D items. This increase is mainly due to regular Focus Group meetings.

The roadmap has also helped Focus Group members to identify an important planning gap: it clearly shows a significant reduction in planned transition and acquisition activity after FY04. This means that several nascent but mission critical technologies may not make it into the hands of the user if suitable transition opportunities are not identified to bring them to the field. The purpose of the Integrated Plan is to ensure that planning for these technologies is based on a strategic vision with a horizon beyond current POM or budget cycles, though detailed funding requirements will not be articulated until a given activity is within the budgetary timeline. Regular Focus Group meetings must therefore continue, in accordance with the new annual process articulated above, in order to proactively articulate R&D community requirements for transitioning critical biodectection technologies to the field and thus ensure adequate and timely funding.

DoD/DOE Integration Strategic Goals and Potential Performance Metrics

The strategic goals of this cross-organizational effort are threefold.

- To foster research and development cooperation and cross-organizational information sharing that results in better CBD products for the users
- To identify and optimize R&D synergies to bring more products to the field and get them fielded faster
- To leverage information and lessons learned to realize the above goals at a lower cost to both agencies.

These goals reflect the fundamental basics for business success—"better, faster, cheaper," or quality, productivity, and cost. Many models exist for measuring performance against these fundamental objectives. The Focus Group will use a balanced measures approach (Figure 5), comprised of both output and outcome measures,[6] to track performance against the strategic goals of RDA integration.

[6] Output measures reflect units or services produced by a program (e.g., the number of coordination meetings held or the number of technology roadmaps produced or updated). The goal of output measures is to verify that a new or established process is being executed as planned. Outcome measures reflect results—the accomplishments achieved by the process (e.g., quality products, faster fielding time, reduced RDA costs).

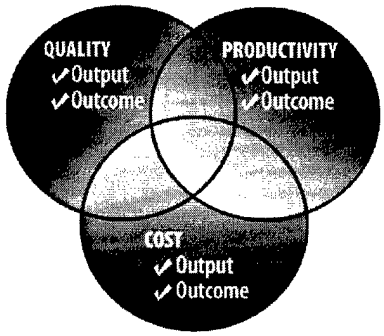


Figure 5. Balanced Measures System

The intent of the Focus Group is to keep measures simple and transparent. Measures are a tool for both the executors of a program and those that oversee it to track its success; they should not be cumbersome or become an end in themselves. The Focus Group has not yet developed the actual measures it will use, but Table 2 provides some potential examples of both process and outcome measures for each of the three strategic goals. These examples serve only to illustrate the different types of measures (output, outcome); the actual measures adopted by the Focus Group may or may not implement any of these examples.

Table 2. Sample RDA Integration Measures

Strategic Goal	Output Measures	Outcome Measures
Better CBD Products	• Number of interagency technical meetings • Percent of technology areas included in roadmaps • Percent of U.S. government and foreign partnership RDA efforts included in roadmaps	• Integration of demonstration and testing activities as a result of roadmapping process • User satisfaction with products tested or fielded
More Products to the Field, Faster	• Increase in testing and demonstration of new technologies • Increase in integrated forward planning over a longer horizon	• Number of products tested/demonstrated each year following integration process initiation (goal is a positive delta; targets TBD) • Decreases in program/project timeline slippage attributable at least in part to integration • Number of products fielded each year • Number of products that have applications for both DoD and DOE users

Table 2. Sample RDA Integration Measures (continued)

Strategic Goal	Output Measures	Outcome Measures
Increased RDA Cost Efficiency/Effectiveness	• Increase in integrated testing and demonstration • Number of occasions where information sharing allows fledgling efforts to move higher on a learning curve	• Decreased cost overruns • Products fielded/individual program cost • Reductions in overall FTE per product fielded (cross-agency view that reflects leveraging of expertise and knowledge)

Clearly some of the sample measures shown in Table 2 are more practical to implement, and some provide more definitive assessments of progress against objectives. In addition, some can be adopted early as the integration process becomes established, while others are not practical until integration is more complete across relevant agencies, technology areas and users. Process measures are most useful early, to help ensure that a new process is becoming institutionalized. Thereafter, while a few process measures need to be retained, outcome measures become more important. Each year, the Focus Group will select measures for the following year at the annual spring meeting at which investment decisions are made.

Conclusion

The Focus Group members believe that their efforts to foster CBD RDA integration between DoD and DOE have yielded important progress over the past year. First, the group achieved its twin goals of developing an integration roadmap template for integrated planning, using biological point detection as the pilot technology area, and developing an annual process for creating and updating the roadmaps. Second, the nascent integration process has already achieved some important initial successes, notably in the areas of interagency coordination in mass spectrometry research and development and in interagency knowledge leveraging in detection on a chip. Finally, the cooperative planning process has identified the need to address the significant gap in FY05 and beyond of opportunities to transition biodetection technologies now under development to users who need those technologies to meet their WMD mission driven security needs. Because of these successes, and others we anticipate based on this year's experience, the Focus Group believes it will be valuable to replicate the process used to develop the biological point detection roadmap across other technology areas. We look forward to taking the necessary action to do so, and will report our progress next year.

APPENDIX A

Acquisition/Transition Activities Involving Biodetection Technologies

The transition and acquisition activities to which biological point detection research and development programs and DoD and DOE make significant contributions are introduced below. They include three EMDs (JPBDS, JCBAWM, and JMCBD), three DOE operational technology demonstrations (PROTECT, BASIS, and Bioforensics), and several types of testing and validation programs. The DoD activities, as well as the supporting R&D programs, contribute to requirements developed in two key pieces of guidance: the Joint Field Operational Capabilities (JFOCs) list and the Areas for Capability Enhancement (ACEs) identified as necessary to the achievement of the JFOCs.

Engineering and Manufacturing Development (EMD) Programs

Joint Biological Point Detection System (JBPDS) EMD

The JBPDS program will provide a common integrated biological point detection suite for use by all services. It will be used to protect air bases, ports, ships, and forces. It will automatically detect and identify 10 Biological Warfare (BW) agents. The focus of **JBPDS Blk I** is automation and increasing the number of BW agents identified. **JBPDS Block II** efforts are centered on decreasing system size, weight, and power and increasing system sensitivity. The program timeline is FY98-FY06. The roadmap shows possible candidate R&D systems for block II transition. These include: BSPS-PCR, BSPS-ESI/MS, Bio-ToF MS, Immunobead Force Differentiation Assay, and Optical Particle Classifier.

Joint Chemical Biological Agent Water Monitor (JCBAWM) EMD

The JCBAWM program is a far-term program that will provide an automatic sensor system to detect the presence of chemical and biological contaminants in potable water. The Army, Air Force, and Marines have identified this requirement. A tech base program is underway. The program timeline is FY05-FY09. The roadmap shows several candidates for transitioning to this program. These candidates are the Advanced Multifunction Biochip, Activity Based Detection and Diagnostics and μChemLab.

Joint Modular Chemical and Biological Detector (JMCBD) EMD

The JMCBD program goal is to generate a detection device that can be used alone or in networks and can identify both chemical and biological agents. This system begins EMD in FY08. Technologies that may be used in this system include Biological Time of Flight Mass Spectrometer, Advanced Multifunction Biochip, μChemLab, Pyrolysis-GC/Ion Mobility Spectrometry and Optical Particle Classifier. There is also some supporting R&D in Aerosol Samplers that should contribute to this program.

Domestic Demonstration and Application Programs (DDAP)

Program for Response Options and Technology Enhancements for Chemical/Biological Terrorism (PROTECT)

The PROTECT program is focused on developing and deploying early CB agent detection, identification and warning (DI&W) systems for vulnerable, heavily populated civilian facilities such as subway systems and airports. The program timeline is FY00-FY04. A review of the roadmap demonstrates the various sensor system R&D technology candidates for this program. These candidates include: APDS, Gene Chip Biosensor, Advanced Multifunction Biochip, μChemLab, PY-GC/IMS, and OPC.

Biological Aerosol Sentry and Information System (BASIS)

The BASIS program is focused on developing early DI&W systems for limited duration bio-agent aerosol monitoring during special events such as major sporting events and political conventions.

The program timeline is FY00-FY02. A review of the roadmap shows CBNP Nucleic Acid Based Assay program deliverables contributing to the highly selective and sensitive detection element of BASIS.

Bioforensics

The purpose of the Bioforensics program is to transition DOE bioforensic capabilities from the laboratory into the hands of intended users: law enforcement, the judiciary, public health, and national security. These capabilities consist of a spectrum of DNA-based techniques that will help the user address a number of bioforensic challenges such as recognizing and documenting a bioterrorist attack and distinguishing it from natural disease outbreak. The timeline of the program is FY01-FY04. The roadmap illustrates CBNP Nucleic Acid Based Assay program deliverables as candidate bioforensic tools for this program.

Test/Validation

Critical Reagents Program (CRP)

The CRP was created by the Joint Program Office for Biological Defense (JPO-BD) in order to ensure security and availability of standardized high quality antibodies, antigens, and gene probes and primers for biological warfare detection systems. In addition, the CRP is responsible for the production of the HHAs, which are the identification components in many existing biological detection systems as well as DoD Biological Sampling kits. CRP timeline initiates at FY98 and has no termination. Both CBNP Nucleic Acid Based Assay and CBDP Reagent Development R&D programs are developing candidate reagents for the CRP.

Joint Field Trials (JFT)

The purpose of the JPO-BD JFT program is to evaluate new and existing technologies for incorporation into biological defense programs. JPO-BD sponsors a JFT test once a year in which developers provide test items that are evaluated by analysis teams. Successful technologies are subsequently matured for integration into detection systems. The program timeline begins in FY98 and has no termination. The majority of Sensor/System R&D program items take place in JFT testing at some time (see roadmap).

DNA Reagents Testing and Validation

This program is responsible for testing and validating DNA-based assays and reagents that are developed in the DOE Nucleic Acid Based Assays Sensor/System R&D Program. The two programs together represent an effort that partially addresses the DOE Biological Foundation program. The roadmap indicates that products from the R&D program will be taking part in the DNA Reagent Testing and Validation process in 4QFY01, 1QFY03, and 1QFY04.

Miscellaneous Testing/DoD

This program identifies the possibility of various testing opportunities to take place when projects arise. There is no current specific target associated with this process. Testing occurs on an as needed basis.

Miscellaneous Testing/DOE

Like DoD, DOE's Miscellaneous Testing program, in addition to JFT and DDAP, also provides test opportunities for projects as they arise. Examples include wind tunnel and ECBC testing.

Guidance

Areas for Capability Enhancement (ACEs)

The ACEs process was established by the Counterproliferation Program Review Committee (CPRC). This process defines priority areas where additional capabilities are needed to meet the challenges induced by NBC weapon proliferation and delivery. A detailed list of each ACE and its designated target area can be found in the *CPRC Report on Activities and Programs for Countering Proliferation and NBC Terrorism*. There is one ACE that addresses detection, identification, characterization and warning of CBW agents; these biological point detection programs support that ACE. The ACEs timeline is unlimited.

Joint Future Operational Capabilities (JFOC)

JFOC was established by the Joint Service Integration Group in an effort to identify and prioritize Joint User far-term future operational capabilities as expressed in the emerging Joint NBC Defense Concept. The overall intent is to provide enhanced user guidance to the Joint NBC Defense Science and Technology (S&T) community to assist in the NBC S&T program formulation and execution process. Prioritized JFOCs include:

- Contamination Avoidance
- NBC Battle Management
- Collective Protection
- Restoration Capability
- Individual Protection

A detailed description of JFOC can be found in the NBC Defense Annual Report. The JFOC timeline is unlimited. (Note that Point and Standoff Detection are included within the JFOC definition of Contamination Avoidance.)

Biological Detection and Identification: Current Programs and Projects

Sensor/System R&D programs include Biological Point Detection and Identification, Reagents/Assay Development, and Supporting Technologies. Biological Point Detection and Identification Programs are further subdivided into major activity areas: Genetic Detection, Detector on a Chip, Mass Spectrometry, and other programs that have not yet been categorized.

Genetic Detection

Autonomous Pathogen Detector System (APDS): DOE CBNP

The LLNL APDS is a stand-alone instrument designed to provide automated, continuous monitoring of aerosols for detection and identification of potential biological agents. Major components include an aerosol collector, sample preparation module, flow cytometer, and Polymerase Chain Reaction (PCR) thermocycler. The system is presently being designed to utilize a combination of both multiplex immuno-based flow cytometer and genetic recognition (via PCR) assays.

Biological Sample Prep System DTO-PCR (BSPS): DoD CBDP

The ECBC BSPS is an automated sample processing system that has the capability to lyse and process spores, bacteria, and virus samples. Lysis methodology is still being optimized; however, down-select processes have shown the Cepheid bead-based ultra-sonication to be the current method of choice for the genetic platform. The processed sample is characterized by a Taqman-based PCR. Nucleic acid detection reagents against eight bacterial and viral agents are in development.

Detector on a Chip

Gene Chip Biosensor: DoD CBDP

The Gene Chip Biosensor, under development at ECBC, has as its objectives to first individually develop and then to demonstrate proof-of-principle integration of two DNA technologies that will offer an enhanced capability over current methods to detect and identify bacterial and viral bioagents, at the strain level, in samples of unknown composition. The two technologies are "universal" PCR amplification and DNA Microarray ("gene chip") analysis. The PCR will use a "universal" random primer set and fluorophore-nucleotide conjugates to amplify and label all DNA present in a sample. Species and strain-level identification of the amplified genetic material will be carried out through the use of a DNA microarray. This work will also begin to integrate the two technologies for use in a complete DNA detector.

Advanced Multi-Function Biochip (AMB): DOE CBNP

The AMB is a fully integrated fluorescence-based microelectronic device developed by ORNL in collaboration with Becton Dickinson and Honeywell. AMB capabilities include bioassay multiplexing generated by engineering different types (DNA, antibody, enzyme) of bio-receptors on the same chip. Genetic and immunologic assay systems include Strand Displacement Amplification (SDA) and Enzyme-Linked Immunosorbent Assay (ELISA) methodologies, respectively. Aerosol collection and sample processing will be provided by mesopump- and ultrasound-based technologies.

Argonne MAGIChip: DoD DARPA

The Argonne MAGIChip is a microchip sensor being developed for the identification of pathogenic organisms. MAGIChip biomolecular reactions take place in a polyacrylamide gel matrix that provides a controllable, 3-D liquid phase environment in which multiple analysis may be performed. MAGIChip capabilities include identification of both RNA and DNA targets, toxin proteins, strain mutations, PCR amplification, and distinguishing between alive and dead organisms. The chips can be regenerated and used several times. This technology is easily amenable to automation.

Activity-Based Detection and Diagnostics: DoD DARPA

This program is being developed to demonstrate that living cells and tissues can be engineered to detect biological and chemical threats. These cell/tissue-based biosensor systems could potentially provide dramatic new capabilities for sensing the activity of existing, emerging, and engineered biological and chemical warfare threats or hazards. The approach is to extract cell/tissue agent response signatures from living systems and ultimately put these signatures on a chip platform.

Mass Spectrometry

Biological Time of Flight Mass Spectrometer: DoD DARPA

The Bio-ToF MS is being developed at JHU-APL for the detection of aerosolized bio-agents, including bacteria, virus, and toxin threats. It utilizes a unique sample ionization process called Matrix-Assisted Laser Desorption Ionization. The Bio-ToF is also capable of automatic aerosol collection as well as automatic sample transport and processing (See Appendix B for more details).

Biological Sample Prep System DTO-ESI/MS (BSPS): DoD CBDP

The BSPS is an automated sample processing system that has the capability to lyse and process spores, bacteria, and virus samples. Biomarkers from the sample are separated by high performance liquid chromatography (HPLC) and subsequently subjected to electrospray ionization mass spectrometry (See Appendix B for more details). Protein mass spectral databases against eight bacterial and viral agents are in development.

Advanced Ion Trap Mass Spectrometer: DOE CBNP

ORNL is developing a mass spectrometer system that will provide for simultaneous detection and identification of bio-agent protein targets. Proteins were the target of choice due to their ubiquitous nature in each biological threat category: bacteria, virus, and toxin. The technique utilizes an electrospray/Ion-Ion chemistry process that facilitates mass spectra analysis of proteins (See Appendix B for more details). This spectrometer program has the potential to leverage off the DoD CBMSII program, developed for the Army.

Science and Engineering Services Incorporated (SESI) Infrared Mass Spectrometer: DoD DARPA

The SESI mass spectrometer has been uniquely designed to identify biological agents. The system utilizes an infrared and ultraviolet laser desorption ionization process for sample ionization. This process generates more signature masses than conventional ionization methods, which provides a higher level of certainty in bioagent identification. This is an important capability, especially when considering spore forming bacteria. A commercial SESI instrument is presently available that has already undergone a spore signature study and will be tested against live agents in the near future.

Other

μChemLab/CB: DOE CBNP

SNL and ORNL are developing a hand portable system for detection of chemical and biological agents. The technical approach utilizes micro-machined chips that contain parallel and serial micro-separation columns/channels. The μChemLab is composed of two main subsystems: (1) A liquid phase module is used to detect biotoxin and other biomolecular signatures and (2) The gas phase module is used for chemical agents. The liquid module utilizes parallel separation channels on the chip followed by UV-fluorescence-based detection, whereas the gas module uses a combination of gas chromatography channels coupled with an array of surface acoustic wave sensors.

Up-Converting Phosphor Flow Cytometer (UCPFCM): DoD DARPA

The SRI UCPFCM is a compact UCP-diode laser-based flow cytometer being developed to detect biological agents and meet detection requirements for JBPDS Block II. Because there are many spectrally unique phosphors activated by the same energy, this system has the ability of multiplexing. Further supporting the use of UCP technology is that UCP compounds are easily detected in dirty environments, which provides for a highly sensitive, low false alarm rate system.

Up-Converting Phosphor Handheld Assay (UCPHHA): DoD DARPA

The SRI UCPHHA utilizes the same UCP technology as the UCPFCM. The primary objective of this project is to evaluate UCP technology in the U.S. Government standard hand held assay (HHA) format using Government Furnished Equipment (GFE) antibodies. A secondary objective is to develop a hardened handheld biosensor that incorporates UCP-based HHA strips for field operation. Research is already underway in modifying the standard HHA with UCP technology. A Multi-target Lateral Flow Wick Assay has been developed that has demonstrated multiple target identification in the same assay.

Immunobead Force Differentiation Assay (FDA): DoD CBDP

The FDA, under development at the Naval Research Laboratory (NRL), is a highly specific and sensitive biosensor capable of measuring antibody-antigen bond forces using magnetic immunobeads. Goals include identifying bacteria, viruses, and toxins with one agent-containing particle per liter of air (ACPLA) sensitivity and greater than 99 percent specificity in less than 15 minutes.

Pyrolysis-Gas Chromatography/Ion Mobility Spectrometry (PY-GC/IMS): DoD CBDP

The PY-GC/IMS is a sensor being developed for both chemical and biological detection. The effort examines the potential for discriminating biological materials at a level of classification higher than "bio" versus "non-bio". This is accomplished by GC/IMS analysis of chemical markers produced upon pyrolysis of biological materials. IMS is already employed in fielded detectors for chemical agents.

Optical Particle Classifier: DoD CBDP

The Optical Particle Classifier, under development at NRL, is an effort to improve the performance of optical trigger systems. This will be accomplished through exploration of optical parameters, including angular elastic scattering, in addition to fluorescence to differentiate biological particles from other materials that fluoresce. Key parameters being evaluated are particle size and shape as well as fluorescence on individual particles.

Reagents/Assay Development

Reagent Development (Antibodies and Alternatives): DoD CBDP

This effort is being performed by a number of laboratories. The focus is to explore and utilize genetic recombinant techniques for the production of specific antigen-binding antibody fragments to antigens of high priority in biological defense. Biased libraries, generated from immunized animals, or unbiased random combinatorial libraries serve as the principal supply of antibody clones. At present, the major focus is on biased libraries. Candidate recombinant antibody fragments are implemented in ELISA, HHA, and other immuno-biosensor platforms for comparison of efficacy with established reagents. Candidates showing high potential are submitted to the Critical Reagents Program (CRP) for validation and employment in fielded sensors. Ongoing efforts in this program are taking place at a number of locations including, ECBC and NMRC.

Nucleic Acid Based Assays: DOE CBNP

This R&D effort is part of the DOE Biological Foundations program. The overall objective of Biological Foundations is to provide an integrated body of biological information and tools as a foundation for CBNP. Expected nucleic acid-based capabilities generated from these programs include: (1) Development of tools and methods for rapidly identifying and isolating unique DNA in an organism to, over time, reduce the cost and time of signature development by more than a factor of 100. (2) Production of whole-genome DNA sequence data for key pathogens and their nearest neighbors as a resource for signature development. (3) Development of informatics tools to facilitate the development, sharing, utilization and archiving of pathogen DNA sequence signatures. Nucleic Acid-Based Assays developed in this program will be subsequently tested in the DNA Reagents testing and Validation, Critical Reagents, BASIS, and Bioforensics Acquisition/Transition Activity programs.

Supporting Technologies

Ambient Background Characterization: DoD CBDP - DOE CBNP

Ambient background characterization is an effort to collect representative background samples as well as to develop a set of heuristics describing the background that may be encountered by detectors in field application. The project is a joint CBDP-CBNP task in collaboration with The Technical Cooperation Program (TTCP) member countries and leverages the prior collection of background data from various sites around the world by a number of programs. The project is scheduled as a two-year effort culminating in FY01 with planned follow-on to collect additional data to fill identified gaps.

Aerosol Samplers: DoD CBDP / DOE[7]

Basic aerosol technology provides a capability to generate and characterize standard test aerosols and CB simulant aerosols in the field and in laboratory facilities—including chambers and wind tunnels. This aspect of the aerosol technology program is focused on quantitative analyses of aerosols to provide the contamination avoidance commodity area with systematic quantification of developmental aerosol collectors and their inlets, in order to accelerate the hardware development process. It also provides well-characterized aerosol challenges to support standoff detection development. Near-term investments are being implemented in a wind tunnel capability for a wide range of challenge aerosols at speeds up to 60 mph. A second area of emphasis is aerosol collector technology. This includes the design of improved aerosol inlets processing elements such as ducts, concentrators, and size-selective devices (*e.g.*, impactors and cyclones), and collection devices for the aerosol particles.

Threat Agent Characterization: DoD CBDP

Investments are being made in the characterization of the properties of threat agents. Emphasis is also placed on developing appropriate simulants for use in the RDT&E process. Execution and funding of the work are integrated across Non-Medical, Medical, and DOE performers and coordinated with the Intelligence Community. Deliverables from this program are technical data on threat agents and simulants for developmental and operational testing.

Threat Agent Characterization: CBNP

In order to improve detection, identification and forensics capabilities, CBNP has begun large collections of several strains of threat pathogens. The *B. anthracis* strain collection is among the world's largest. The program has initiated collaborations with USAMRIID, Rocky Mountain Laboratories, the CDC and British laboratories to expand the collections of strains and closely related organisms.

[7] DOE funding not included in the CBNP budget.

APPENDIX B

Integration Example: DoD and DOE Biological Mass Spectrometry Programs

The Chemical Biological Defense programs of the Departments of Energy and Defense have investments in several efforts to develop biological mass spectrometry for field applications. The following provide a background on the state of biological mass spectrometry, a description of the efforts conducted under these programs, an analysis for duplication and complementarity, and steps taken toward maximizing leverage among the several projects. Figure 6 shows DOE and DoD CBD mass spectrometry program integration efforts.

Background on Biological Mass Spectrometry

Mass spectrometry provides perhaps the most definitive means of identifying chemical species due to the relative ease with which ions can be generated and then manipulated in a mass analyzer to provide accurate mass information for both the intact molecular species and structurally significant fragments of the molecule. Using fundamental principles developed over the last fifty years, the molecular structure of an analyte molecule can be developed purely from the mass spectrum, given the appearance of sufficient data from the observed spectrum. For the general case where insufficient information can be extracted from the observed mass spectrum to build a structure from first principles, identification is made against standard libraries of chemical species developed over the past four decades. For the most general case where the analyte is in a mixture with other chemical species, standard separation techniques (e.g., gas chromatography) have been developed to separate the components prior to ionization and mass analysis. Thus analysis of volatile and semi-volatile chemical unknowns by gas chromatography-mass spectrometry is a mature and reliable technology used both in the lab and in the field.

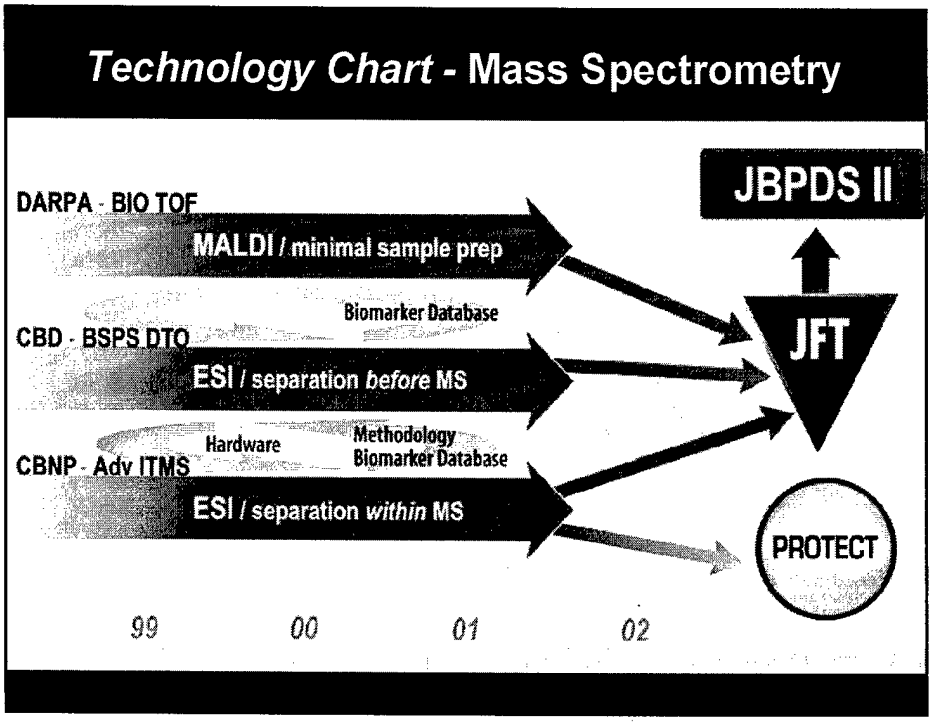
Application of mass spectrometry to analysis of biological materials has similarly great potential for both laboratory and field use. In contrast to biological mechanisms for identification of biological entities, such as immunological reactions and genetic-based identification, mass spectrometry requires no agent-specific reagents and is applicable across the full range of agents of biological origin using a single assay methodology. Additionally, mass spectrometry can develop a signature of an unknown material not previously in its database. Finally, as noted above, mass spectrometry is already accepted as a method of choice for identification of chemical materials. Thus, a single identification platform based on mass spectrometry can be applied to both chemical and biological threats.

There is, however, greater texture in the development of mass spectrometric methods for analysis of biological materials than for chemical materials.

- Biological materials present a generally significant mixture analysis challenge even when a single species is present: for example, a single bacterium comprises greater than a thousand distinct chemical species. Currently there exist a number of methods to address this mixture analysis challenge.
- A number of different molecular classes, whose members may constitute markers that serve to identify a material, are found in the full spectrum of agents of biological origin, including proteins, lipids, and nucleic acids. Each class will be best analyzed by a different approach.
- Biological molecules have generally high mass, have no volatility, and are fragile to heating, requiring special approaches to generate gas phase ions as required for mass spectrometry. Major developments in the past decade have identified and matured two

viable approaches to sample handling: matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI).

- Observed mass spectra are dependent on a number of factors, hence, databases will iterate as these factors are understood until the optimum database is developed.



- This chart summarizes the Mass Spectrometry Project Review. The text inside the colored arrows indicates the method of mass spectrometry and the sample handling differences between the different approaches. This situation is complementary, rather than duplicative, and is ideal because the "right" answer is not yet known.
- These projects are actually benefiting from each other; the arrows between the projects show where they are sharing databases or hardware, or both.
- DARPA, CBD and CBNP projects go through the JFT to transition to JBPDS Block II.
- The CBNP project is designed for use in the DOE PROTECT DDAP (the DoD projects might also participate if the testing done in PROTECT will be beneficial to execution of the programs).

Figure 6. Programs Included in the Mass Spectrometry Project Review

Biological Mass Spectrometry Projects in DoD/DOE

Each of the major players in BW defense science and technology (DoD Joint Services CBDP, DARPA BWD, DOE CBNP) has an investment in biological mass spectrometry. Additionally, through The Technical Cooperation Program, interface with projects in the UK CBD program exists to further leverage U.S. investments. UK CBD also invests in development of biological mass spectrometry.

The following are brief descriptions of the four major investments in biological mass spectrometry research and development within the CB defense community.

DoD Joint Services Chemical Biological Defense Program

Defense Technology Objective CB20 (Biological Sample Preparation System) seeks to develop and demonstrate by FY01 an automated sample processing system incorporated with mass spectrometric analysis. The goal is to demonstrate a system that automatically analyzes samples collected from air into liquid. The systems will automatically extract biomarkers from sample, clean up and separate them, and submit the separated biomarkers for analysis in a mass spectrometer. The analysis will be conducted by electrospray ionization mass spectrometry and tandem MS/MS of protein biomarkers separated by high-performance liquid chromatography, prior to introduction into the mass spectrometer. Proteins extracted from biological samples may also be subjected to digestion to produce characteristic peptides. Protein mass spectral databases against eight bacterial and viral materials will be delivered. Target capabilities include detection at one agent ACPLA in 15 minutes. Target acquisition program insertion is JBPDS Block II in FY02.

DOE Chemical Biological National Security Program

The goal of this project (Advanced Ion Trap MS) is the development of a fieldable prototype electrospray ion trap mass spectrometer for the rapid detection and identification of biological threats in a civilian, counterterrorism scenario. The analytical approach for this project is the analysis of intact proteins. A novel ion-ion chemistry approach that allows the reduction or elimination of many slow, complex sample preparation or separation steps is being developed to simplify mass spectra from multicomponent mixtures by reducing ion charge states within the mass spectrometer. When combined with protein sequence information from the tandem MS/MS analysis, the technique allows the rapid identification of these proteins (and thereby the host organism if the protein is unique) using protein databases. The combination of the ion storage capabilities in an ion trap with the ion selection and charge state manipulation potentially allows the isolation of target proteins within the ion trap analyzer. This process reduces the need for extensive "front-end" sample cleanup, digestion, or pre-analysis separations. The target product of this program is a fieldable mass spectrometer identification system complete with automated (intended for non-expert users) sample preparation, analysis and data interpretation capabilities.

UK DERA Chemical Biological Defense Program

The goal of this project is to develop a fieldable biological mass spectrometer based on electrospray ionization mass analysis of protein biomarkers from agents of biological origin. Protein biomarkers are to be extracted from samples collected into fluid from air, with various approaches to sample cleanup being investigated. A primary emphasis is placed on exploration of immunoaffinity-based cleanup for the targeting of specific materials (either biomarkers or entire antigens) to concentrate from a mixture prior to subjecting to mass analysis. The application of enzymatic digestion as part of the sample processing prior to mass analysis is also explored as a means of increasing discrimination through structural information. Proteomic databases are envisioned to provide specific biomarkers to target for detection.

DARPA Biological Warfare Defense Program

DARPA BWD has invested in the development of an automated MALDI mass spectrometer system for the near-realtime analysis of biological particles collected from air onto a sample substrate. Particles in the air sample are impacted onto a tape and co-deposited with matrix required for the ionization process. The tape with sample is moved into the ionization region of the mass spectrometer, where a low power laser beam illuminates the sample and product ions whose mass is determined in a time-of-flight mass spectrometer (Bio-ToF MS). Target biomarkers are those of proteins associated with agents of biological origin. Databases are under development. The current focus of the project is to determine essential parameters of the system by exercising it against agents combined with varying concentrations of ambient background collected around the world.

Analysis for Duplication and Complementarity

In all projects described above, the target biomarkers fall in the class of proteins, since proteins are found in all agents of biological origin. These projects can be arrayed into two groups based on type of ionization approach used. The DARPA investments are in MALDI, whereas the other three are focused on ESI.

Among the ESI-based efforts, the essential discriminating feature is in sample preparation, particularly the processes used to separate protein biomarkers and decrease complexity of mass spectra. The DoD JS CBDP effort takes the lowest risk approach by utilizing liquid chromatography to separate protein biomarkers *before* they are admitted to the mass spectrometer. The DOE CBNP effort explores reducing sample processing steps by using ion-ion reactions *within* the mass spectrometer to simplify the analyte spectra in a mixture *after* it is admitted to the mass spectrometer. The UK CBD effort is unique by virtue of developing methodologies based on immunoaffinity to improve pre-ionization sample cleanup by concentrating target classes of molecules on a column designed to capture them and allowing other materials in background to pass.

Each of these three approaches is quite different in addressing the challenge of sample cleanup and biomarker separation. They are in fact complementary, since a system can be envisioned to comprise *all* these approaches. Hence, the characteristic features of these different approaches address different steps in the overall process for discriminating biological materials by mass spectrometry.

Another area of significant complementarity is the development of the biological mass spectral database. All efforts described utilize proteins as the target biomarker class. Different approaches may yield different subsets of the full set of proteins expressed by a bacterium. For example, it will be useful to know which approaches yield the broadest set of proteins, as well as which proteins consistently appear regardless of approach or other conditions. Such "conserved" proteins may serve as the most reliable biomarkers. Hence, the development of a comprehensive data set compiled from all available information is of significant value to all participants in the development of biological mass spectrometry.

Development of Cooperative Effort

The principal investigators (PIs) on these projects are generally aware of each other's efforts through journal articles and through informal discussions as opportunities arise. However, knowledge of results of work prior to publication can lead to six months or so acceleration in related efforts. Additionally, there are many aspects of scientific work that are beneficial to the overall success of an approach but that do not appear in the literature.

In order to increase the leveraging of the efforts identified above, the PIs were called together for a meeting (during the Joint Services Conference on Point Detection, 23-27 Oct 00) to discuss areas of potential sharing of information and mechanisms to effect such information sharing. The PIs reached

broad agreement to share information in a number of key areas. It was agreed to share data and lessons learned on methodologies for sample collection, release of protein biomarkers, sample cleanup and separation, and proteolytic digestion. Additionally, methodologies for production of ions and for automation of sample preparation were areas where PIs felt they could and should share information. Finally, the PIs agreed to share information on biomarkers for various target materials and methodologies for selecting biomarkers. As a component of the biomarker database, database search algorithms were also identified as an area for cooperative development.

Potential issues that might cause the cooperative effort to stumble were identified. Of significant concern was protection of intellectual property. The group leader was charged with investigating need and mechanism for safeguards of intellectual property. It was also noted that as biomarkers for threat agents are identified, this information would become classified. Such information would have to be handled by appropriate channels and mechanisms.

Agreement was reached for this group of PIs to meet at least annually to present and share results of their efforts. For mass spectrometry PIs, such a meeting can be arranged around the annual meeting of the American Society for Mass Spectrometry.

APPENDIX C

Congressional Language Calling for the Integration Effort
Senate Armed Services Committee Language, S. Rpt. 106-50 S. 1059

"In 1996, the CPRC recommended that the Department of Defense and the Department of Energy establish an integrated research, development, and acquisition plan for technologies associated with chemical and biological counterproliferation. To date, there has been no visible result of this CPRC recommendation. The committee directs the Under Secretary of Defense for Acquisition and Technology to submit the integrated plan to the congressional defense committees, not later than March 1, 2000."

Senate Armed Services Committee Language Requiring a Report on CPRC Integration with Domestic Response Users

"In 1996, Congress added a mission to the CPRC charter requiring efforts to '...negate paramilitary and terrorist threats involving weapons of mass destruction.' Given this responsibility, and the resources and expertise available to the CPRC, the committee believes that the CPRC should consider establishing a mechanism for working with the domestic response program to help ensure that the research, development, and acquisition of equipment for domestic response to weapons of mass destruction has appropriate involvement from the user community. The committee directs the CPRC to provide a report to the congressional defense committees, not later than March 15, 2000, on this recommendation and its potential benefit to the domestic response program."