RDT&E BUDGET ITEM JUSTIFICATION SHEET (R-2 Exhibit)								DATE February 1999		
APPROPRIATION/BUDGET ACTIVITY RDT&E, Defense-wide BA2 Applied Research					R-1 ITEM NOMENCLATURE Biological Warfare Defense PE 0602383E, R-1 #14					
COST *(In Millions)*	FY 1998	FY 1999	FY 2000	FY 2001	FY 2002	FY 2003	FY 2004	FY 2005	Cost To Complete	Total Cost
Total Program Element (PE) Cost	58.452	84.754	145.850	151.000	151.500	135.800	116.800	113.800	Continuing	Continuing
Biological Warfare Defense Program BW-01	58.452	84.754	145.850	151.000	151.500	135.800	116.800	113.800	Continuing	Continuing

(U) Mission Description:

(U) The Biological Warfare Defense program is budgeted in the Applied Research budget activity (BA-2) because its focus is on the underlying technologies associated with pathogen detection and remediation. Today, there is a tremendous mismatch between the magnitude of the biological warfare threat and the Department's ability to adequately respond. The widespread availability of bacterial, viral, and toxin stocks; minimal developmental cost and scientific expertise required; and abundance of weaponization potential comprises a sinister threat. The single largest concern, however, is from the exploitation of modern genetic engineering by adversaries to synthesize "super pathogens." Recent dramatic developments in biotechnology, which this program will leverage, promise to eliminate this mismatch. This program funds projects supporting revolutionary new approaches to biological warfare (BW) defense and does not duplicate efforts of other government organizations.

(U) Efforts to counter the BW threat include developing barriers to block entry of pathogens into the human body (including unique methods for rapid air and water purification), pathogen countermeasures to stop pathogen virulence and to modulate host immune response, medical diagnostics for the most virulent pathogens and their molecular mechanisms, biological and chemically-specific detectors, and consequence management tools. Program development strategies include collaborations with pharmaceutical, biotechnology, government, and academic centers of excellence.

(U) Pathogen countermeasures (e.g., Anti-Virals/Immunizations, Anti-Bacterials/Anti-Toxins, Multi-Purpose, and External Protection) under development include: (1) multi-agent therapeutics against known, specific agents and (2) therapeutics against virulence pathways shared by broad classes of pathogens. Specific approaches include modified red blood cells to sequester and destroy pathogens, modified stem cells to detect pathogens and produce appropriate therapeutics within the body, identification of virulence mechanisms shared by pathogens, development of therapeutics targeting these mechanisms, efficacy testing in cell cultures and animals, and advanced non-toxic decontamination strategies.

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(U) In the early stages, many illnesses caused by BW agents have flu-like symptoms and are indistinguishable from non-BW related diseases. Early diagnosis is key to providing effective therapy. The advanced diagnostics efforts will develop the capability to detect the presence of infection by biological threat agents, differentiate them from other significant pathogens, and identify the pathogen even in the absence of recognizable signs and symptoms (when the pathogen numbers are still low).

(U) The ability to detect biological warfare agents on the battlefield in real time with no false alarms is a crucial requirement. To address this need, the program is creating more efficient and effective miniature sampling technologies that concentrate contaminated air and enhance the ability to capture biological warfare agents. The program is developing a new range of antibodies and "designer small molecules" to bind specific agents (to replace the lower affinity antibodies currently used). In order to detect that the binding of an agent has occurred, the event must be "magnified." Traditionally, this is done by tagging the antibody molecule with a fluorescent probe. This program is replacing the noise-plagued fluorescent tags with Up-Converting Phosphors with the sensitivity to detect a single binding event, minimizing the size of the sample required, saving time, and decreasing the number of false positive alarms. The use of fluids as a requirement for biological agent detection is also being eliminated and replaced by a miniaturized (shoe box-size) time-of-flight mass spectrometer. Development of a bacterial biochip to identify genus and species without multiplying the DNA by the polymerase chain reaction (PCR) is also under development, thereby saving at least 20 minutes in time to identification. Additional efforts are focusing on the construction of molecular, cellular, and multicellular sensors for the rapid detection of biological threats. These cellular and tissue-based sensors have the ability to respond to both known and unknown threats, determine live vs. inactivated threat status, and report functional consequences of exposure (mechanisms of action).

(U) Mission effectiveness requires rapid, correct medical responses to biological weapon threats or attacks. This project will provide comprehensive protocols to protect or treat combatants by using current and emerging biological countermeasures. It will provide accelerated situational awareness for biological warfare events by detecting exposure to agents through an analysis of casualty electronic theater medical records and will locate and determine the most effective logistical support for providing appropriate treatment and pathogen-specific resources required to mitigate effects of the attack.

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(U) Program Accomplishments and Plans:

(U) FY 1998 Accomplishments:

- Pathogen Countermeasures. ($ 43.086 Million)
  - Optimized the detection of specific pathogens by stem cells (in cell culture).
  - Determined the impact of modified red blood cells on the vascular and immune systems.
  - Defined animal models in which to test the efficacy of modified red blood cells to defend against pathogens.
  - Developed enzymes and other active molecules which can be attached to the surface of red blood cells to detect and destroy pathogens.
  - Established a portfolio of strategies to:
    - Inhibit the expression of disease causing (virulence) factors by pathogens.
    - Disrupt the disease causing (virulence) communications between pathogens.
    - Modulate the body's response to the presence of a pathogen.
    - Assess the feasibility of novel polymeric materials to protect against pathogen exposure.
  - Assessed the feasibility of an array-based instrument (and other novel technologies) for multi-agent pathogen diagnosis in medical samples.

- Sensors. ($ 7.788 Million)
  - Developed a hierarchical database of mass signatures for use in detecting selected bacteria with a mass spectrometer.
  - Investigated methods for determining biological warfare agent bacterial and viral viability (agent live or dead).
  - Demonstrated the feasibility of using giant magnetoresistance for the detection of magnetic bead-tagged pathogens.
  - Fabricated and tested a wick device, an integral sample pump, and a reagent reservoir system suitable for use in a handheld Up-Converting Phosphor detector.
  - Developed a biochip for rapid pathogen identification.
  - Engineered cells for pathogen specificity and amplification.
  - Engineered cells with optical signals in response to pathogen detection.
  - Identified limiting performance variables for cells in tissue based detection schemes.

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- Consequence Management. ($ 7.578 Million)
  - Demonstrated a biological warfare Anchor Desk that provides agent-specific biological warfare (BW) situational awareness, decision and execution support with linkages to the Logistics Anchor Desk (LAD) for BW-specific logistical information.
  - Developed agent-specific "software antibodies" for detection, protection, and treatment directives to medical personnel for biological warfare (BW) threats that will decrease response time.
  - Developed quantitative measures of operational assessment using Medical Readiness Indicators (metrics based indicators of individual and unit level readiness) and realistic BW training algorithms to improve the medical response to a biological warfare incident.
  - Demonstrated Enhanced Consequence Management Planning and Support System (ENCOMPASS) during BIO 911 and other exercises for command and control of biological warfare incidents.

(U) **FY 1999 Plans:**

- Anti-Virals/Immunizations. ($ 14.820 Million)
  - Develop a modified stem cell, which can both detect and produce a prophylactic/therapeutic response to a pathogen (in cell culture).
  - Determine (in-vitro) toxicity of modified stem cell-produced therapeutics.
  - Create techniques to rapidly develop immunization strategies against bacterial and viral pathogens and toxins.

- Anti-Bacterials/Anti-Toxins. ($ 14.858 Million)
  - Develop and test (in-vitro) cellular platforms for toxin destruction and toxin binding decoys.
  - Demonstrate selected strategies (in cell culture) to:
    - Inhibit the expression of disease causing (virulence) factors by pathogens.
    - Disrupt the disease causing (virulence) communications between pathogens.
    - Modulate the body's response to the presence of a pathogen.

- Multi-Purpose. ($ 12.000 Million)
  - Define animal models in which to test the efficacy of modified stem cells to prevent disease.
  - Demonstrate in laboratory animals the efficacy of modified red blood cells to eliminate pathogens from the blood for the purpose of potential defense against BW agents.

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- Determine pathogen detection and elimination efficacy for modified red blood cells with enzymes or other active molecules attached to their surfaces.

- External Protection. ($ 7.214 Million)
  - Develop polymeric materials for pathogen protection.
  - Demonstrated in-vivo broad-spectrum efficacy of non-toxic biological decontamination formulation.

- Advanced Diagnostics. ($ 10.800 Million)
  - Determine appropriate bodily sample types (blood, saliva, sputum, etc.) to use for diagnosis.
  - Determine which non-biological warfare (BW) pathogens must be screened against because they mimic early symptoms of known BW threat agents.
  - Begin identification of probes to be used in diagnosis systems.
  - Evaluate the feasibility of novel technologies and sampling strategies, such as detecting bodily responses indicative of infection.

- Sensors. ($ 15.462 Million)
  - Continue development of air sampling technology for airborne biological materials.
  - Determine chemotaxonomic biomarkers for selected viral substances for detection in the mass spectrometer.
  - Demonstrate replacement of a surface-bound antibody with a “designer” small molecule for high affinity pathogen capture.
  - Develop high affinity monoclonal antibody that recognizes only anthrax spores without cross-reactivity with vegetative cells (or other bacillus species) and test in existing BW sensors for improved performance.
  - Complete Up-Converting Phosphors (UCP) detection system and field test.
  - Modify the prototype of a miniature biodetection system following Dugway Proving Ground test results.
  - Select cell and tissue types for the development of tissue based sensors.
  - Examine and select strategies to stabilize cell systems for long-term shelf life and functional response.
  - Demonstrate the ability to modify the duty cycle of a cellular response in single cell and tissue based sensors.
  - Demonstrate performance limits of a single cell sensor.

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- Consequence Management. ($ 8.600 Million)
  - Develop software toolkit for Enhanced Consequence Management Planning and Support System (ENCOMPASS).
  - Perform additional field tests of BW defense attack response planning tool and electronic watchboard.
  - Develop electronic watchboard architecture and biological warfare (BW) incident playbook authoring and maintenance tools.
  - Transition BW Medical Readiness Indicators to the Services.

- Multimedia/Telemedicine. ($ 1.000 Million)
  - Develop enhanced telemedicine capability for war fighter by augmenting/tailoring wireless communication technology appropriate for responses to biological warfare attacks.

(U) FY 2000 Plans:

- Anti-Virals/Immunizations. ($ 20.500 Million)
  - Identify broad-spectrum strategies with potential for immunomodulatory activity against multiple pathogens.
  - Develop a method of mucosal immunization based upon high level expression of pathogen antigens and epithelial transport molecules in edible transgenic plant products.
  - Develop technologies for rapid design and development of new vaccines against novel pathogens.
  - Demonstrate (in-vitro) candidate anti-viral and anti-bacterial small molecule therapeutics for selected targets.
  - Demonstrate (in-vivo) the efficacy of anti-viral peptides derived from hematopoietic stem cells.

- Anti-Bacterials/Anti-Toxins. ($ 18.300 Million)
  - Develop (in-vitro) broad spectrum, superantigenic, anti-toxin antagonists and vaccines.
  - Validate the efficacy (in-vivo) of antagonists to toxin receptors, toxin catalytic sites, and cellular platforms for toxin destruction.
  - Demonstrate (in-vivo) toxin-blocking antibodies and toxin binding decoys.
  - Demonstrate (in-vivo) the efficacy of a broad-spectrum bacterial antagonist.
  - Use gene-shuffling techniques to generate molecules to be screened for superantigenic properties.

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- Multi-Purpose. ($ 20.000 Million)
  - Explore concepts for therapeutics against bioregulators and other mid-spectrum agents.
  - Identify primary harmful immune responses to BW agents.
  - Explore concepts for optimizing human immune response to biological warfare (BW) agents, minimizing negative sequelae.
  - Demonstrate in laboratory animal models the ability of modified stem cells to prevent disease.
  - Develop synthetic polymer complements for pathogenic antigens and virulence factors.
  - Identify monomeric and dimeric DNA and RNA binding molecules as novel countermeasures against multiple pathogens.
  - Identify polyvalent inhibitors for inhibiting pathogens on the surface of target cells in-vivo.

- External Protection. ($ 18.500 Million)
  - Develop decoy molecules that will prevent the adhesion of multiple pathogenic toxins or viruses in-vivo.
  - Demonstrate (in-vivo) a non-specific surfactant agent to neutralize biological threat agents.
  - Demonstrate initial performance of a prototype device for the purification of water contaminated with BW agent simulants.
  - Explore high throughput methods for the purification of contaminated air.
  - Demonstrate effectiveness of specific personnel protective toxin and pathogen neutralization strategies against virulent biological agents.
  - Continue development of prototype protective system and initiate integration into personnel protective systems.

- Advanced Diagnostics. ($ 18.700 Million)
  - Continue identification and development of probes to be used in diagnosis systems, and begin testing of probe panels in the laboratory.
  - Develop sample preparation techniques to optimize speed, accuracy, and reliability of diagnosis.
  - Identify one or more promising strategies for rapid detection based on bodily responses or other biomarkers to provide early indication of infection or exposure (including non-invasive early detection of disease [e.g., NO in exhaled breath]).
  - Determine range of cytokine levels in the healthy body verses an infected body using laboratory animals and cell cultures as models.
  - Determine feasibility of engineering red blood cells to detect and signal pathogen presence in the body.
  - Determine feasibility of rapid single molecule DNA sequencing for accelerated patient diagnosis.
  - Explore concepts for diagnosing patients for bio-regulator and other mid-spectrum agent attack.

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- Sensors. ($ 33.850 Million)
  - Complete, test, and verify first-generation prototype of live agent biochip sensor.
  - Complete development of air sampling technology for airborne biological material.
  - Continue development of effective and rapid chip-reading capability with enhanced sensitivity.
  - Continue the development of unique signatures for bio-agents in mass spectrometry identification.
  - Develop biosensor technology for next-generation (bioengineered) threat agents.
  - Develop methods for identifying bioregulator-based biological warfare (BW) agents.
  - Evaluate chemical clues used by biological systems in normal hunting strategies to revector the biological systems to search for BW agent production or storage.
  - Explore options (e.g., training, genetic engineering, etc.) for the use of invertebrates in the detection of BW agents and associated chemicals.
  - Construct cell and tissue engineered configurations to enhance optical or electrical signal output from the sensor.
  - Optimize electronic interfaces for optical and electrical reporting from cell and tissue based sensors.
  - Investigate optimal system designs for deployment of a single cell and tissue based biosensor, which incorporate environmental sampling, microfluidics, and automated detection.
  - Evaluate cell and tissue based informatics from temporal and spatial signals in cell and tissue-based sensors.
  - Explore shelf-stabilization strategies for cells and tissues.
  - Develop bio-agent sensors for use in building protection.
  - Develop capability to predict flow of airborne bio-agents in and around buildings.

- Genetic Sequencing of Biological Warfare Agents. ($ 4.000 Million)
  - Develop inventory of DoD-relevant BW agent pathogens requiring sequencing.
  - Determine best methods for rapidly sequencing biological warfare pathogens and related species and strains.
  - Begin development of database mining techniques to find new targets for sensors, diagnostics, and therapeutics.

- Consequence Management ($ 12.000 Million)
  - Develop distributed BW consequence management smart checklists for automatic pull and push of required information.
  - Continue development of Enhanced Consequence Management Planning and Support System (ENCOMPASS) software toolkit.

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- Continue development of playbooks and incorporate Incident Command System capabilities.
- Demonstrate use of ENCOMPASS for OCONUS air base force protection against a BW attack.
- Demonstrate use of playbooks and automated checklists for training BW incident responders.

(U) FY 2001 Plans:

- Anti-Virals/Immunizations. ($ 21.300 Million)
  - Validate (in-vivo) a method of mucosal immunization based upon high level expression of pathogen antigens and epithelial transport molecules in edible transgenic plant products.
  - Test and validate (in-vivo) the protective efficacy of vaccines and antibodies produced by plant cells against pathogens.
  - Demonstrate the efficacy of the rapid and efficient delivery of pathogen antigens via new genetic vaccine vectors.
  - Demonstrate (in-vivo) the rapid design and development of new vaccines (or therapeutics) against unidentified or unknown pathogens.
  - Demonstrate broad-spectrum strategies with potential for immunomodulatory activity against multiple pathogens.

- Anti-Bacterials/Anti-Toxins. ($ 19.500 Million)
  - Demonstrate surface expression of specific enzyme molecules for the rapid inactivation of various pathogens.
  - Demonstrate (in-vivo) the efficacy of a broad-spectrum bacterial pathogen antagonist.
  - Validate (in-vivo) broad spectrum, superantigenic, anti-toxin antagonists and vaccines.
  - Demonstrate (in-vivo) efficacy of broad spectrum, superantigenic, antitoxin antagonists and vaccines.

- Multi-Purpose. ($ 22.100 Million)
  - Develop therapeutic strategies against bioregulators and other mid-spectrum agents.
  - Demonstrate synthetic polymer complements for pathogenic antigens and virulence factors.
  - Develop therapeutic strategies for minimizing harmful immune responses to biological warfare agents.
  - Demonstrate (in-vitro) the efficacy of monomeric and dimeric DNA and RNA binding molecules as novel countermeasures against multiple pathogens.
  - Validate polyvalent inhibitors for blocking pathogens on the surface of target cells in-vivo.
  - Identify superantigens for broad protection against biological warfare agents with minimal side effects.

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- Validate (in-vivo) the efficacy of subcellular pathogen response imaging for rapid detection.
- Validate technologies broadly applicable to enhance cellular therapeutics (delivery platforms) and virulence modulation (intracellular and inflammatory cascades).

- External Protection. ($ 20.600 Million)
  - Develop a novel architectural approach for the manufacture of materials that are effective in blocking pathogens and limiting disease.
  - Demonstrate a non-aqueous advanced decontamination method.
  - Demonstrate scalability of a water purification system effective against a range of biological agents (including toxins and bioregulators).
  - Build and test a prototype air purification system for individual soldiers.
  - Begin testing of prototype protective system against non-virulent biological warfare (BW) agents.
  - Begin testing of prototype protective system against bio-toxins and bio-regulators.

- Advanced Diagnostics. ($ 21.000 Million)
  - Test probe panels in relevant sample types including strategies for rapidly generating new/novel probes.
  - Demonstrate that sample collection and/or preparation techniques do not introduce artifacts.
  - Test, in model systems, one or more of the most promising candidate strategies for rapid detection based on bodily responses or other biomarkers to provide early indication of infection or exposure.
  - Develop the capability to diagnose exposure to bio-regulator and mid-spectrum agents.
  - Demonstrate, in the laboratory, the feasibility of engineering red blood cells to detect and signal pathogen presence in the body.
  - Evaluate the feasibility of a strategy for detection of disease using exhaled breath.
  - Evaluate the feasibility of additional strategies for direct identification or detection of infection without direct sample collection.
  - Demonstrate the ability to perform accelerated patient diagnosis using a rapid single molecule DNA sequencing technique in a model system.

- Sensors. ($ 34.000 Million)
  - Continue the development of effective and rapid chip-reading capability with enhanced sensitivity and low false alarm rate.

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- Continue the development of advanced alternative technologies for live vs. dead bio-agent identification using peptides and other molecules.
- Evaluate methods for removing micro-encapsulation of disguised pathogens and/or sensing through the micro-encapsulation.
- Continue the development of technologies required for next-generation miniature biological detectors including the use of microelectromechanical systems (MEMS), microfluidics, and mesoscopic-sized components.
- Evaluate false positive and false negative rates for systems of detectors.
- Exploit and/or mimic the olfactory sensors of biological systems for use in the detection of biological warfare agents.
- Engineer a deployable prototype cell and tissue sensor for field-testing.
- Demonstrate enhanced signal output from engineered cells and tissue based sensors.
- Integrate information from cell and tissue sensors with user interfaces for predictive responses.
- Develop concepts for sensors capable of detecting biological warfare agent production in underground facilities.
- Investigate critical design parameters for advanced biologically based BW sensor.
- Validate bio-agent sensors for use in building protection.
- Continue development of capability to predict flow of airborne bio-agents in and around buildings.
- Determine optimal sensor placement for building protection.

- Genetic Sequencing of Biological Warfare Agents. ($ 2.500 Million)
  - Continue development of database mining techniques and test on a subset of pathogenic genomes.
  - Transition sequencing activity to Department of Energy funding.

- Consequence Management. ($ 10.000 Million)
  - Demonstrate rapid construction and distribution of specific BW smart checklists for multiple responders.
  - Demonstrate Enhanced Consequence Management Planning and Support System (ENCOMPASS) management of multi-site BW incidents.
  - Demonstrate automatic construction of incident- and responder-specific playbooks and electronic watchboards.
  - Demonstrate use of ENCOMPASS for CONUS air base force protection against BW attacks.
  - Transition ENCOMPASS to National Guard Rapid Assessment and Initial Detection Units and to Air Force Theater Battle Management Core.

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(U) Program Change Summary: (*In Millions*)

	FY1998	FY1999	FY2000	FY2001
Previous President's Budget	60.805	88.000	77.300	74.000
Current Budget	58.452	84.754	145.850	151.000

(U) **Change Summary Explanation:**

FY 1998	Decrease reflects SBIR reprogramming and minor program repricing.
FY 1999	Decrease reflects net effect of congressional program and undistributed reductions; congressional add for BW telemedicine demonstration and below threshold reprogramming.
FY 2000/01	Increases reflect Departmental direction to expand biological warfare efforts in the following areas: biological agent detectors; enhanced medical diagnostics and therapeutics; air and water purification technologies; and genetic sequencing of biological warfare agents.

(U) Other Program Funding Summary Cost:

- Not Applicable.

(U) Schedule Profile:

- Not Applicable.