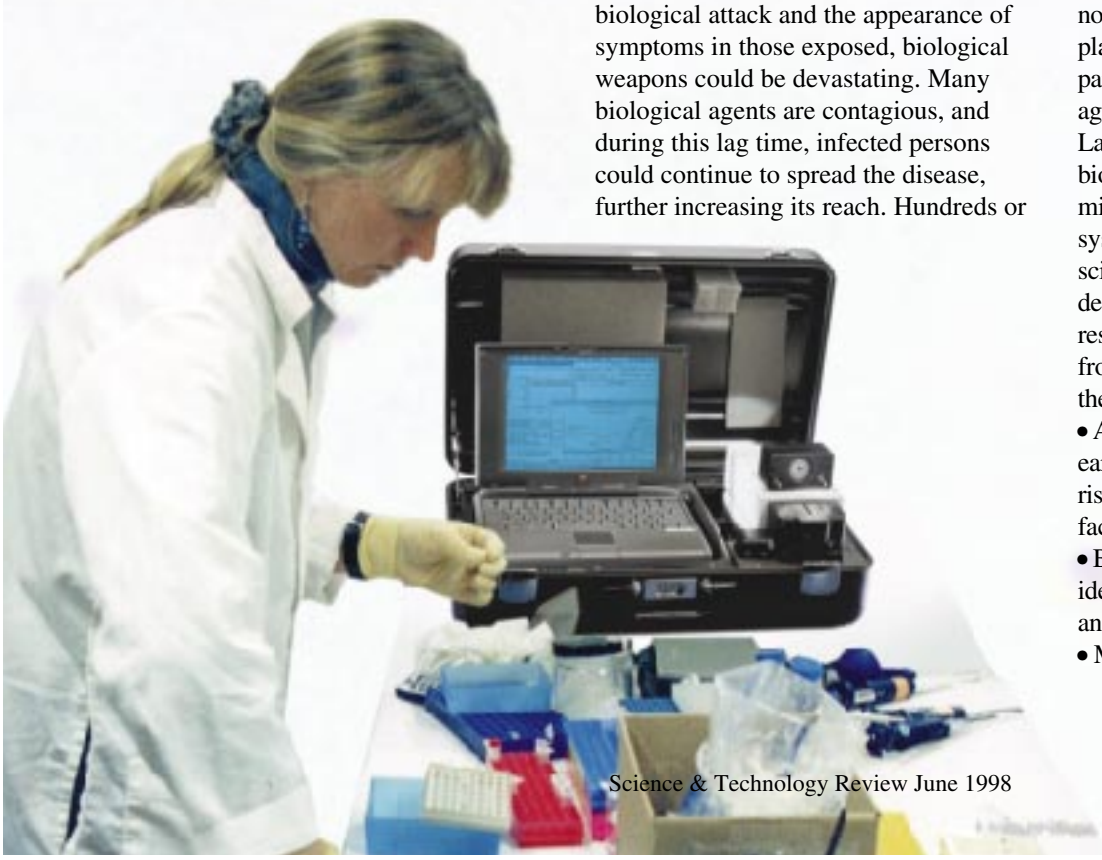
Reducing the Threat of Biological Weapons

Livermore's strategy for defense against the use of biological weapons integrates technology, operations, and policy and provides a framework for coordinated local, state, and federal emergency response.

"WEAPONS of mass destruction" is a terrifying term. We all have mental images of the horrors of a nuclear attack, and photos of Kurdish and Iranian casualties of Iraqi chemical attacks attest to the devastation of chemical weapons. The third weapon of mass destruction—the biological weapon—has been around at least since the Middle Ages when soldiers catapulted the bodies of dead smallpox victims over fortress walls in the hope of infecting their enemies or at least demoralizing them.

Lately, biological weapons have been appearing in the news with increasing frequency. The anthrax threat in Las Vegas in February of this year is an example. Surplus stores in Las Vegas sold out of gas masks, and talk-radio shows were swamped with callers asking about evacuation points. That threat turned out to be a false alarm, but the next one might be real.

Biological agents are of concern in part because of the ease with which many of them can be manufactured, transported, and dispensed. And because of the lag time between a biological attack and the appearance of symptoms in those exposed, biological weapons could be devastating. Many biological agents are contagious, and during this lag time, infected persons could continue to spread the disease, further increasing its reach. Hundreds or even thousands of people could become sick or die if a biological attack were to occur in a major metropolitan area.



With the knowledge that several nations have produced and perhaps also deployed biological warfare agents, Congress in 1996 passed the Defense Against Weapons of Mass Destruction Act, which authorizes the Department of Energy to establish a Chemical and Biological Weapons Nonproliferation Program. Under this and similar programs, Lawrence Livermore and other laboratories and institutions are working together to increase this country's capabilities to detect and respond to an attack by biological or chemical weapons.

Beginning as recently as Fiscal Year 1996 with a Laboratory Directed Research and Development strategic initiative, Livermore has rapidly expanded its chemical and biological nonproliferation program and is now playing a lead role in this effort, particularly as it pertains to defense against biological weapons. The Laboratory is applying its investment in biological science, engineering, microtechnology, computer modeling, systems analysis, and atmospheric science to a number of programs designed to improve the country's response to a biological attack. Personnel from departments and directorates across the Laboratory are at work on:

- Advanced detection systems to provide early warning, identify populations at risk and contaminated areas, and facilitate prompt treatment.
- Biological forensics technologies to identify the agent, its geographical origin, and/or the initial source of infection.
- Methods for predicting the transport

of biological agents in urban environments and for assessing the area and duration of the hazards associated with a biological attack.

• New decontamination technologies to clean and restore facilities without causing further environmental damage.

Livermore is working closely with the U.S. military, various government agencies, and such major cities as New York City and Los Angeles to ensure that the results of these biological nonproliferation efforts meet the needs of military troops, the FBI, local law enforcement personnel, fire fighters, public health officials, and others who would likely be first on the scene following a biological attack. Together these groups are answering questions to help create the best, most task-appropriate, and most usable system possible. For example, how accurate do sensors have to be? What level of false alarms can be tolerated? Where will sensors be located—in buildings, on emergency response personnel, or at other sites? How much training will be feasible for emergency response personnel on the use of sensors and decontamination agents—that is, how user-friendly must these processes be?

Livermore is developing a strategy for defense against the use of biological weapons that integrates technology, operations, and policy and provides a framework for coordinated local, state, and federal emergency response.

Better Detection Systems

A key factor limiting the nation's ability to protect against a biological attack has been the state of biodetector technology. Only now is technology becoming available that permits identification of biological organisms within minutes, when concentrations are low but often still dangerous. Before the revolutions in genomics, biotechnology, microengineering, and microcomputers, such identification could only be done in a laboratory and took days to weeks. Soon, however, technology advances—many of them made at Lawrence Livermore—will offer the possibility of rapid, accurate, and sensitive biodetectors for use in battlefield or urban settings.

Figure 1. Ray Mariella, Jr., working with a multichambered PCR (polymerase chain reaction) unit. In the 1997 Advanced Concept Technology Demonstration, this PCR instrument proved an effective tool for field identification of the DNA in nonvirulent bioagent simulants.

Automation Is Key

Livermore is developing two types of fully automated biodetectors for real-time sample collection, detection, and identification in the field. A miniature flow cytometer (known as miniFlo) uses an immunoassay system to look at the proteins and other material on the surface of cells, and a portable PCR (polymerase chain reaction) unit identifies the DNA inside the cell. (See the box on p. 6 for more information on these systems.) Because of their small size and efficiency, both units process data much faster than their laboratory-scale cousins, while maintaining the highest level of sensitivity.

To fully automate sample collection and preparation, Livermore is developing and testing components for an aerosol biocollector and a microfluidic sample preparation system. The device will collect and sample particles in the air, including biological agents, if present. To maximize detection potential and give faster results, the PCR unit and miniFlo are also being "multiplexed" to handle multiple samples at once. Other system improvements are being made to both instruments to lower the rate of false positives (false alarms), increase the sensors' sensitivity, and make the systems even smaller, more rugged, and less reliant on consumables than they are now. Livermore expects to have continuously operating, integrated biosensors available for use within the next few years.

With two types of sensors working in tandem, the chance of false alarms will be reduced considerably. Tolerance for false alarms differs greatly for military versus civilian situations. Deployed troops are already in a state of heightened readiness, with protective equipment available and the training required to react to attack situations. In contrast, with civilians, false alarms could lead to injuries and perhaps to dismissal of future legitimate alarms. Thus the military may be able to afford some level of false alarms, but the goal for the civilian sector is no false alarms.

The miniFlo and the PCR systems have proved their mettle against established performance criteria at the U.S. Army's international Joint Field Trials at the Dugway Proving Grounds in Utah. At Dugway, participants use a variety of instruments to detect simulant materials representative of typical biological weapon materials.

At the 1996 Joint Field Trials III, miniFlo was superb at detecting *Bacillus globigii* and *Erwinia herbicola* (nontoxic simulants for anthrax and plague respectively) at various low concentrations. Overall, miniFlo detected 87% of all unknowns with a false alarm rate of under 0.5%. At the 1997 Port/Airbase Advanced Concept Technology Demonstration and the January 1998 Dugway Joint Field Trials IV, the portable PCR unit clearly demonstrated the potential of PCR as an effective technique for field identification of DNA (Figure 1).

Networked Detectors

A networked system of these or other biodetectors could provide U.S. troops in the field with early warning of a biological attack. That is the goal of a project for the Department of Defense known as JBREWS (Joint Biological Remote Early Warning System), on which Livermore is collaborating with Johns Hopkins Applied Physics Laboratory and Los Alamos National Laboratory. As shown in Figure 2, JBREWS will consist of a network of sensors and communication links. By tying this network into the military's existing communications systems, JBREWS will take advantage of well-established command and communications procedures. Initially equipped with commercially available sensors, JBREWS is being configured so that improved biodetectors can be incorporated into the system as they become available.

Livermore is responsible for what is known as "C4I"—command, control, communications, computers, and intelligence. The Laboratory is developing the connectivity between the sensors and the control station, the software for all sensors, and an automatic analysis and reporting system that runs up through the military chain of command. JBREWS is scheduled to be demonstrated in a Department of Defense Advanced Concept Technology Demonstration in 1998.

Biological Forensics at Work

If a bacterium or spore appears in a collected sample, how will a biodetector know what it is? The key to identification will be a library of "signatures" of the makeup, function, and DNA of various biological agents that will be stored on a microchip in the detector, together with pattern-matching software and code for reporting results. This technology will allow advanced detectors in the laboratory and ultimately in the field to quickly match the signatures of collected particles to signatures in its memory, in much the same way that fingerprints are matched.

Building on years of experience in genomics and biotechnology, Livermore scientists are expanding the

Livermore's New Biodetectors

Portable PCR

In late 1996, Lawrence Livermore delivered to the U.S. Army the first fully portable, battery-powered, real-time DNA analysis system. DNA analysis requires many copies of a DNA sample, which are made by the polymerase chain reaction. PCR requires repeated cycles of an aqueous sample being heated close to the boiling point and then cooled. To detect DNA in a sample, a synthesized DNA probe or primer tagged with a fluorescent dye is introduced into the sample before it is inserted into the heater chamber. Each probe or primer is designed to attach to a specific organism—anthrax, plague, etc. If that organism is present in the sample, the probe attaches to its DNA. By measuring the sample's fluorescence, the instrument reports the presence (or absence) of the targeted organism.

In Livermore's portable unit, the thermal cycling process takes place in a micromachined, silicon heater chamber that has integrated heaters, cooling surfaces, and windows through which detection takes place. The PCR reaction and DNA analysis take place in a disposable polypropylene reaction tube inserted into the heater chamber.

Because of the low thermal mass and integrated nature of Livermore's silicon heater chambers, they require very low power and can be heated and cooled much faster than conventional units. So the unit is not only portable but also much faster and more energy-efficient than bench-top models. A multiple-chamber unit that allows the examination of many samples at the same time has been field tested.

MiniFlo

Livermore's miniature flow cytometer is the latest in a series of flow cytometers developed over the past two decades in Livermore's Biology and Biotechnology Research Program Directorate. Flow cytometers are used in laboratories to analyze cells and their features, perform blood typing, test for diseases and viruses, and separate out particular cells or chromosomes. What sets miniFlo apart from other flow cytometers is its small size, portability, and sensitivity.

These features are made possible by a novel system that eases the alignment and increases the accuracy of flow cytometry. In a flow cytometer, the cells flow in single file in solution while the experimenter directs one or more beams of laser light at them and observes the scattered light, which is caused by variations in the cells or DNA. Instead of using a microscope lens or an externally positioned optical fiber as a detector, this method uses the flow stream itself as a waveguide for the laser light, capturing the light and transmitting it to an optical detector. This approach not only eliminates the alignment problems that plague traditional flow cytometers but also collects ten times more light than a microscope lens does. Simpler alignment and more light mean better, faster analysis.

Bacteria are large enough for individual detection in the miniFlo, but viruses and proteins are not. So beads large enough to be detected are coated with an antibody and added to the sample. The virus or protein attaches itself to the bead and can then be detected. When different beads are coated with different antibodies, simultaneous detection of several biological agents is possible.

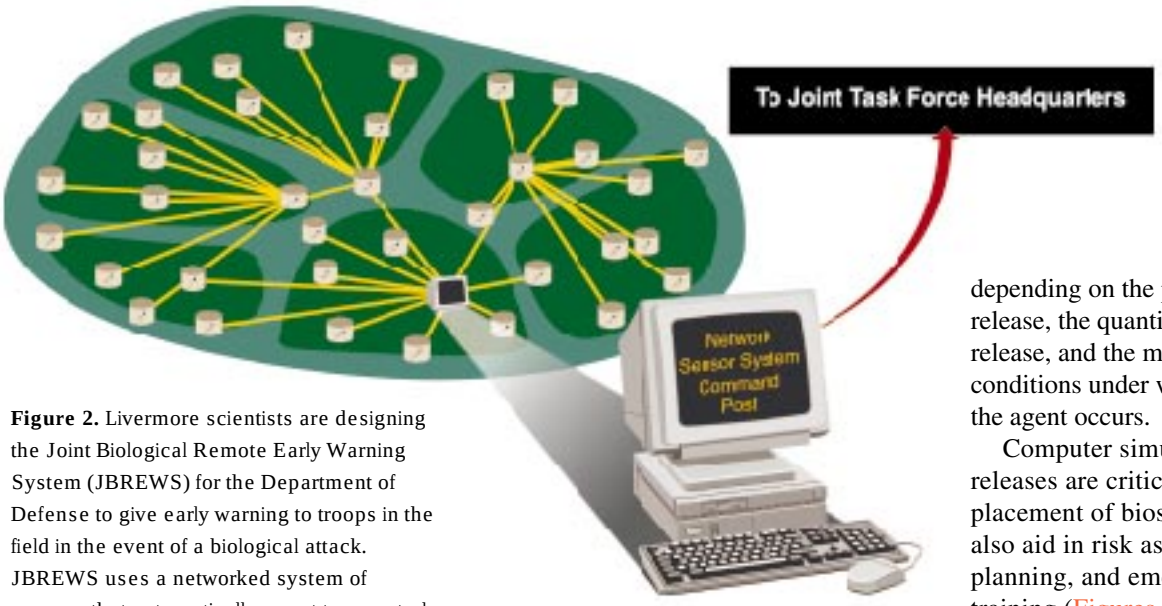


Figure 2. Livermore scientists are designing the Joint Biological Remote Early Warning System (JBREWS) for the Department of Defense to give early warning to troops in the field in the event of a biological attack. JBREWS uses a networked system of sensors that automatically report to a central computerized command post.

information base of the DNA sequences of biological agents to enable rapid, unambiguous identification of biological agents. To facilitate this process, they are developing ways to speed up the process of finding unique DNA sequences among organisms.

A process known as representational difference analysis helps to identify unique DNA sequences. Parts of the DNA of two organisms are mixed. If they stick together, they match; if they do not stick, they are unique parts. Currently, this process is cumbersome and slow, but Livermore scientists are working to automate it to be able to examine many sequences in parallel.

Another project is studying specific pieces of bacterial DNA and examining the possibility of using their location as an indicator of differences among strains. A third project is investigating virulence factors, which are the genes that give a biological organism its infectivity or toxicity. If a bioweapon is being genetically engineered, those genes might be moved to an unnatural host in an attempt to thwart detection and identification.

In addition to identifying the particular agent being used, tools being developed at Livermore also seek to provide information that will help to identify the perpetrator of a biological attack. Livermore biomedical researchers were among the first to study regional differences among the various naturally occurring strains of anthrax and other biological agents. Law enforcement personnel will be able to match data about a pathogen with data on regional or strain characteristics (indicators of engineered characteristics) and with data on worldwide biological research, epidemiology, and infectious diseases and respond to the threat.

Predicting Agent Dispersion

The ability to accurately predict the dispersion, concentration, and ultimate fate of biological agents released into the environment is essential to prepare for and respond to a biological agent release. Of particular concern is the threat to civilian populations within major urban areas where potential terrorist incidents are more likely to occur. There the hazard from a biological-agent release could be confined to a localized area within or around a single building or extend out to a large portion of the city or even into the surrounding suburbs, depending on the particular agent release, the quantity and duration of the release, and the meteorological conditions under which dispersion of the agent occurs.

Computer simulations of biological releases are critical to the design and placement of biosensor systems. They also aid in risk assessment, disaster planning, and emergency response training (Figures 3 and 4). If a biological release were to occur, real-time predictions of agent concentrations would be used to characterize the source, estimate exposure levels, identify affected areas and best evacuation routes, and later assist with decontamination. Accurate information about the likely course of a bioagent attack is key for emergency response managers, who must notify health officials, inform emergency response teams, and make public safety decisions.

The urban biological release problem is quite complex and requires modeling capabilities that are still in the early stages of development and application. For example, models of airflow inside buildings and subways have been developed to some degree but do not accurately incorporate the decrease in airborne concentration that results from deposition of the toxic material on walls, ceilings, ventilation ducts, and other interior surfaces. Similarly, computational fluid dynamics models of the highly distorted flows and dispersion patterns created by complexes of buildings are just beginning to include the effects of biological aerosols (gravitational settling, deposition, and viability degradation) and multiple building interactions.

Lawrence Livermore, Lawrence Berkeley, Los Alamos, and Argonne

national laboratories are working together to develop an integrated and validated atmospheric modeling capability for biological agent releases in an urban environment. They will be applying these models to case studies in a range of release scenarios, from closed office buildings, to subway systems, to stadiums and street corners. The goal is to make the models applicable to real-life situations and ultimately to integrate them into the incident response capability of the National Atmospheric Release Advisory Center, located at and operated by Lawrence Livermore.

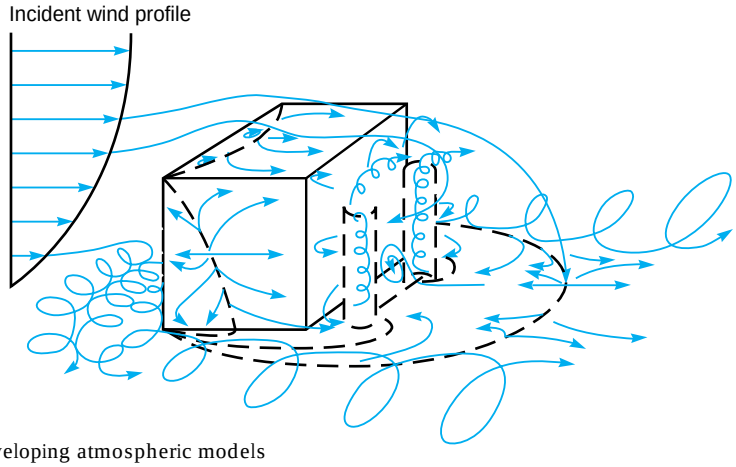


Figure 3. Developing atmospheric models for an urban setting requires taking many flow patterns into consideration. As shown here, air movement around just one building is highly complex.

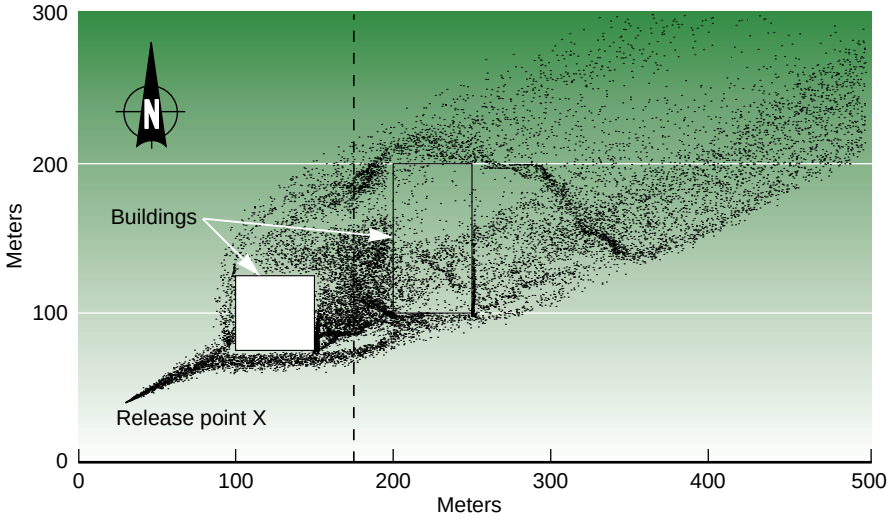


Figure 4. This scenario shows where particles will be 10 minutes after they are released at point X in a 240-degree (west southwest) wind of 10 meters per second. Several areas of high particle concentration are visible to the south of the two buildings, with lesser concentrations to the north and to the east.

Decontaminating a Site

After an area has been exposed to a biological attack, it must be decontaminated before it can be reopened to the public. Livermore and Los Alamos national laboratories are working together to develop decontamination strategies for three scenarios—an open stadium, a semi-enclosed subway, and an enclosed area such as an office or home. Certain decontamination methods might be acceptable for one scenario but not another. For example, more corrosive reagents and large volumes of water might be acceptable in a stadium but could not be used in an office building.

Plain household bleach is one of the best decontamination agents around, and it is used regularly in biological laboratories throughout the country. But 5% sodium hypochlorite (as bleach is more technically known) is a very caustic product, so it must be used with care. The team is working to develop decontamination methods that are as effective as bleach but more acceptable environmentally.

Decontamination proceeds in several stages, from cleanup of gross contamination such as puddles of agent, to localized decontamination of walls or furniture that were directly exposed to the agent, to cleanup of ductwork or inaccessible cracks for hidden contamination, and finally to long-term remediation such as special paints or sorbents to destroy small quantities of agent that are left after completion of other decontamination. These stages may require different cleanup materials. A variety of liquids and powders are being studied, as is an array of delivery methods such as foams and gels. One treatment method that has been found to be effective and more environmentally acceptable than hypochlorite (an alkaline product) is peroxymonosulfate, which is an acidic oxidizer. Figure 5 compares treatment of a simulant for anthrax with these

(a) No treatment (b) Peroxymonosulfate treatment (c) Hypochlorite treatment

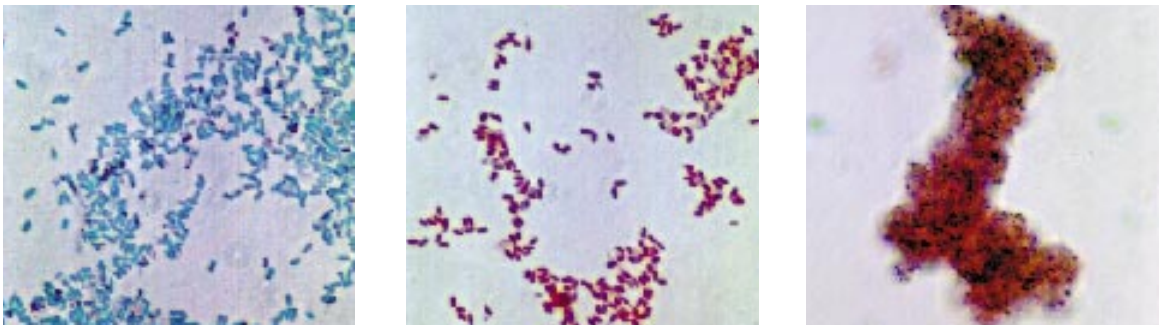


Figure 5. *Bacillus globigii* spores (a simulant of the spores that cause anthrax) are shown (a) before and (b) after a 30-minute exposure at 22°C to peroxymonosulfate, an acid oxidizer, and (c) after treatment with hypochlorite, an alkaline oxidizer. Spores were stained with malachite green (blue-green) and safranin (red) dyes. Safranin dye penetrates only dead spores because of their damaged walls, thus making it a good indicator of the effectiveness of a biocide.

oxides. The selected method must be not only effective but also easy to use with minimal training.

The social and political issues involved in decontamination and reentry to a site are not being overlooked. Central to these concerns is "How clean is clean enough?" The team is coordinating with the biosensor developers to devise sampling and analysis systems that can verify that decontamination is complete.

One hurdle for the decontamination process is that no real-time biodetector currently under development at Livermore uses an assay that can distinguish between viable organisms and dead or decontaminated ones. Work has begun on a "viability assay" based on flow cytometry to provide this important piece of information so that decontamination can proceed in a timely manner.

Responding to the Threat

The threat of biological weapons is all too real, and the U.S. must be prepared to respond if a bioattack occurs on the battlefield or in a civilian setting. During the 1991 Gulf War, the U.S. had no systems available for rapid, timely field detection of bioagents. The situation today is very different. The military has deployed Biological Integrated Detection Systems (BIDS), which can tentatively identify the presence of a suspected biological agent in the field and warn soldiers to take appropriate action to protect themselves against the agent, pending positive laboratory identification. And there are also programs such as Livermore's that include new detection, identification, atmospheric modeling, and decontamination capabilities, which, combined with work by others on better vaccines and medical treatment, are bringing the country to a level of preparedness that can meet a biological threat.

—Katie Walter

Key Words: biodetectors, bioinformatics, biological warfare agents, decontamination, DNA analysis, flow cytometry, genomics, miniFlo cytometer, National Atmospheric Release Advisory Center (NARAC), polymerase chain reaction (PCR), weapons of mass destruction (WMD).

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About the Scientist

FRED MILANOVICH received his B.S. in physics from the United States Air Force Academy in 1967 and a Ph.D. in applied physics from the University of California at Davis in 1974. He is currently program manager for the Chemical/Biological Nonproliferation Program within the Nonproliferation, Arms Control, and International Security Directorate at the Laboratory. This program is providing an integrated response to the emerging threat of chemical and biological terrorism with innovation in detection technology, bioinformatics, fate and transport analyses, and incident response. Milanovich has published extensively in his field and holds many patents for optical sensors and measurement instruments. His research interests also include trace biodetection, laser spectroscopy, analytical instrumentation development, and microtechnology.