Rapid Field Detection of Biological Agents

FOR years, experts in terrorism have been warning that a terrorist attack with biological agents is not a question of "if" but "when." As recent events have proved, when is now.

For almost a decade, researchers at Lawrence Livermore, working on the when-is-now premise, have been developing systems that can rapidly detect and identify biological agents, including pathogens such as anthrax and plague. (For more background on Livermore's research against bioterrorism, see *S&TR*, June 1998, pp. 4–9, and May 2000, pp. 4–12.) Among such systems are the Handheld Advanced Nucleic Acid Analyzer (HANAA) and the Autonomous Pathogen Detection System (APDS).

The Handheld Advanced Nucleic Acid Analyzer can detect biological pathogens in the field.

Lawrence Livermore National Laboratory

Although HANAA and APDS are of different sizes and made for different situations, they have a common purpose: to get results, fast. Lawrence Livermore biological scientist Richard Langlois explains, "There are any number of laboratory tests available right now to analyze pathogens. They all require getting a sample and then transporting it to a laboratory for processing. Our systems use new instrumentation and methods that provide faster and more timely results, on the spot. Faster results mean the responders can act quickly and begin treatment earlier."

HANAA in Hand

About the size of a brick, the HANAA biodetection system can be held in one hand and weighs less than a kilogram. The system was designed for emergency response groups, such as firefighters and police, who are often first on the scene at sites where bioterrorism may have occurred. Each handheld system can test four samples at once—either the same test on four different samples or four different tests on the same sample. HANAA can provide results in less than 30 minutes, compared with the hours to days that regular laboratory tests typically take.

The process of detecting and identifying what's in a sample works like this. The operator prepares the samples by putting them in a liquid buffer and adding chemicals. A tiny disposable plastic tube holding about 0.02 milliliter of the prepared liquid is then inserted into the system. Many copies of a sample's DNA are needed to analyze it and identify its makeup. HANAA uses a technique called the polymerase chain reaction (PCR), which amplifies agent-specific DNA fragments to a detectable level. In PCR, an aqueous sample is heated close to the boiling point and then cooled many times (40 times in HANAA). Every time the DNA is heated, the two intertwined strands of DNA unwind and come apart. As the sample cools down, the DNA makes a copy of itself. Thus, at the end of each cycle, the amount of DNA is doubled.

To detect the DNA in a sample, a synthesized DNA probe tagged with a fluorescent dye is introduced into the sample before it is inserted into the heater chamber. Each probe is designed to attach to a specific organism, such as anthrax or plague. Thus, the operator must have an idea of what substances might be involved. "The system doesn't test for all unknowns," says Langlois. "A responder has to decide what kinds of pathogens to test for ahead of time and set up the system accordingly." If that organism is present in the sample, the probe attaches to its DNA, which is then amplified during the PCR process, releasing the fluorescent tag. HANAA

measures the sample's fluorescence and the presence (or absence) of the targeted organism.

One of the big breakthroughs for the handheld system involved the design of a small silicon heater chamber for the heating and cooling cycle, a concept developed at Livermore by Allen Northrup, a former Laboratory scientist. "The commercial thermocyclers used for standard laboratory tests are pretty big, ranging from the size of a microwave oven to a large desk," notes Langlois. "A typical large thermocycler takes about 3 minutes to cycle through one heating and cooling cycle, so a complete analysis requires 2 to 3 hours." In the HANAA system, the thermal cycling process occurs in tiny silicon heater chambers, micromachined by Livermore's Center for Microtechnology. Each chamber has integrated heaters, cooling surfaces, and windows through which detection takes place. Because of the low thermal mass and integrated nature of the chambers, they require little power and can be heated and cooled more quickly than conventional units. The mini-chambers typically cycle from about 55°C to 95°C and back to 55°C in about 30 seconds.

Using this technique, the HANAA system could, in principle, detect as few as 10 individual bacteria in one-hundredth of a milliliter in less than 30 minutes. The system has the potential of saving many lives by saving time—anthrax, for example, is highly treatable if detected early.

The Laboratory has a cooperative research and development agreement for HANAA with Environmental Technologies Group (ETG), a chemical and biological detector company and subsidiary of Smith's Industries, based in Baltimore, Maryland. ETG expects to have a commercial version of HANAA available early this year. Ron Koopman, special projects manager for the Chemical and Biological National Security Program at Livermore, notes that HANAA is essentially ready to go at this critical juncture because of the forward-thinking efforts begun in the previous decade. "A number of people recognized the vulnerability of the country to bioterrorism a long time ago," he says. "In 1996, although bioterrorism seemed far away and was something we hoped would never happen, the Laboratory and members of the defense community decided to invest in the research, just in case. Thanks to that investment, we now have something to put in the hands of people to protect us all, something that can help during the current crisis and in the long run."

A Bio "Smoke Detector"

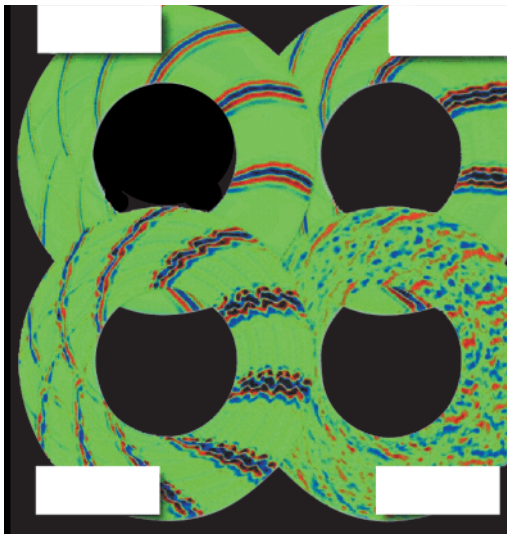
Whereas HANAA can be hand-carried to sites at which an attack is suspected to have happened, the APDS is stationed in one place for continuous monitoring and is designed to work much like a smoke detector, but for pathogens. When fully developed, the APDS could be placed in a large area such as an airport, a stadium, or a conference hall. The system will sample the air around the clock and sound an alarm if pathogens are detected.

"The important point here is that the system would be fully automated," stresses Langlois. "The system will collect and prepare the samples, do the analysis, and interpret the results, all without human assistance."

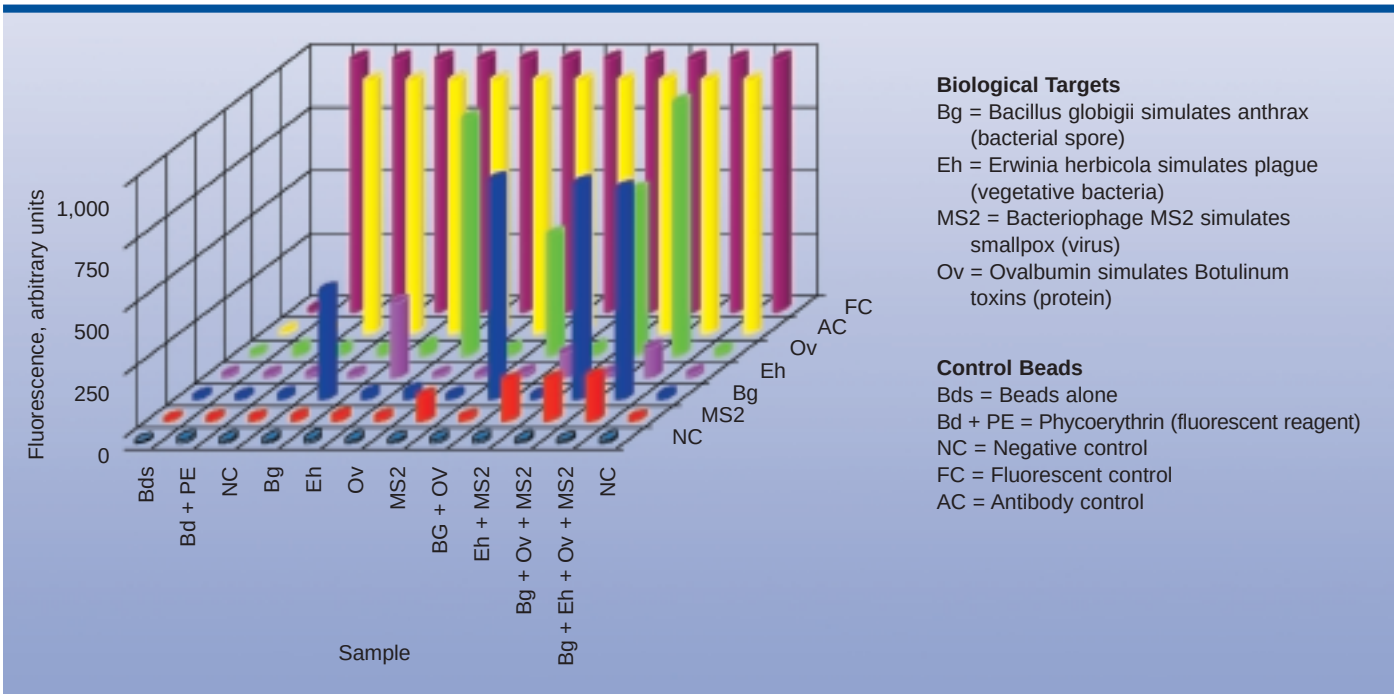
Livermore is testing the second APDS prototype, which is about the size and shape of a lectern or mailbox. The APDS-II consists of an aerosol collector, a sample preparation subsystem, and two subsystems for detecting and analyzing the samples: one based on PCR and the other based on flow cytometry, which uses antibodies to identify pathogens. "The final system will double-test each sample to decrease the likelihood of false positives and increase the reliability of identification," explains Langlois.

The aerosol collector, which was designed by Vern Bergman and Don Masquelier at Livermore, gathers an air sample every 30 minutes—the length of time it takes to complete a sample analysis. A built-in fan pulls in the air, which passes through a glass tube containing water. The water traps any particles in the air, and the resulting fluid is pumped to the next stage for sample preparation and testing.

The flow-through PCR subsystem for the APDS includes a Livermore-designed thermocycler—much like the thermocycler in HANAA—along with a sequential injection analysis system. This analysis system performs all the necessary PCR sample preparation functions, such as mixing the sample with PCR



The Autonomous Pathogen Detection System is capable of continuous, automated, 24-hour monitoring for pathogens, with results reported every 30 minutes.



Results from the flow-cytometry subsystem of the Autonomous Pathogen Detection System (APDS) using seven color-coded "capture" beads coated with antibodies specific to the target pathogens. The pathogens attach to their respective antibodies and then to more antibodies added to the sample mixture that are labeled with fluorescent dyes. When the beads pass one by one through the flow cytometer's laser beam, any bead with labeled antibodies will fluoresce, and the APDS identifies the pathogens present, depending on the color of the capture bead.

reagents, delivers the resulting liquid sample to the thermocycler, and decontaminates the thermocycler chamber and fluid delivery tubes to prepare for the next run.

For the flow-cytometry subsystem, small "capture" beads that are 5 micrometers in diameter are coated with antibodies specific to the target pathogens. The beads are color coded according to which antibodies they hold. Once the pathogens attach to their respective antibodies, more antibodies—those labeled with a fluorescent dye—are added to the mix. A labeled antibody will stick to its respective pathogen, creating a sort of bead sandwich–antibody, pathogen, and labeled antibody. The beads flow one by one through a flow cytometer, which illuminates each bead in turn with a laser beam. Any bead with labeled antibodies will fluoresce. The system can then identify which agents are present, depending on the color of the capture bead. "Right now, we use seven bead types to detect four agents simultaneously with controls," says Langlois. The next step is to increase the number of detectable pathogens to 20 or 30. Ultimately, the researchers expect to be able to test for a hundred pathogens simultaneously in a single assay.

Langlois and the APDS team hope that, within the next year or two, the system will be ready to put in place wherever needed. Ultimately, notes Langlois, numerous detector systems could be linked together in a network connected to an emergency response center to protect a complex of buildings or a city.

The Faster the Better

From handheld, immediate testing to autonomous and continuous testing, HANAA and APDS are two of many systems Livermore is developing to help the nation fight bioterrorism. With HANAA, emergency responders can get answers on the scene in less than half an hour. With APDS, no human direction will be necessary, and the system will perform on its own, completely self-contained, monitoring 24 hours a day, 7 days a week. "What ties these approaches together is the ability to analyze a sample quickly—within 30 minutes or less—and do it on site," concludes Langlois. "Getting the answer quickly is important. In the case of a biological attack, the sooner we know what bioagent we're dealing with, the sooner treatment can start for those affected. Systems such as these have the potential for saving many lives."

—Ann Parker

Key Words: anthrax, Autonomous Pathogen Detection System (APDS), biodetectors, biological warfare agents, bioterrorism, DNA analysis, flow cytometry, Handheld Advanced Nucleic Acid Analyzer (HANAA), pathogens, polymerase chain reaction (PCR).

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