To prepare for the next bioterrorist attack, researchers say they need faster and more accurate ways to detect the spread of deadly microbes

Biodefense Hampered by Inadequate Tests

The recent spate of anthrax attacks plaguing the United States has not just created panic but also strained public health labs to their limits. Many thousands of tests have been performed literally around the clock to monitor the spread of the disease and prevent further deaths—and yet the public has found the results often confusing and sometimes contradictory.

Many people in America's new hot zones became worried about flulike symptoms, potentially the first signs of inhalation anthrax, and wanted to get tested—only to find out that they couldn't. Buildings that tested positive—such as a Microsoft office in Reno, Nevada—were later declared safe, or vice versa.

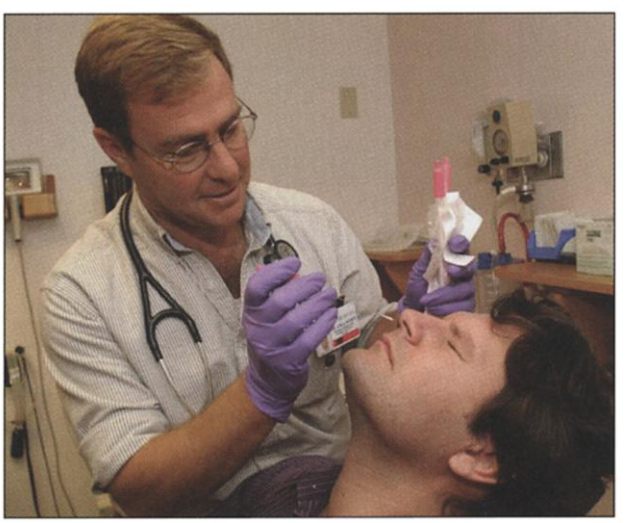
But scientists say such problems are unavoidable: The arsenal of tests they have to work with falls short, they say, forcing them to strike an uncomfortable balance between speed and accuracy. "It's obvious that we need new diagnostics" to detect biowarfare pathogens, both in the environment and in the human body, says Jim Hughes, director of the National Center for Infectious Diseases in Atlanta, part of the Centers for Disease Control and Prevention (CDC). Diagnostics are needed "not just for bioterrorism candidates but also for the diseases that they're easily confused with," he adds. Such tests need to combine three features that current diagnostics often don't have: speed, accuracy, and ease of use.

Now, prodded by the unnerving events of the past 4 weeks, government and industry labs are stepping up efforts to develop tests that can detect the presence of anthrax and tell whether a person is infected. Experts predict a tremendous push to develop many new tools in the coming years. Indeed, companies at the forefront of pathogen detection find themselves among the few that are doing very well since 11 September.

Tests and trade-offs

As the four deaths to date from inhalation anthrax illustrate, this bacterium can kill very quickly, and there are no tests to reliably determine early on whether somebody has either been exposed to it or been infected. Thousands of people have undergone nasal swabs. But although such sampling provides an idea of how far contamination has spread within a building, it says little about a person's risk. Even if spores are absent from the nose, they may be present in the lungs, waiting to germinate; likewise, finding spores in a nasal swab indicates exposure but not necessarily infection.

Even during the initial phase of the disease, when flulike symptoms such as fever, malaise, or nausea occur, there's no easy way to locate the germs within the body. They might be present in the lungs or lymph nodes, but to detect them would require invasive and painful procedures. Researchers are working on better ways to spot infection—for instance, by looking for signs in patients' breath—but those are a long way off.



Nosing about. Nasal swabs can show whether a person was exposed to anthrax spores; they do not indicate infection.

In the absence of such tests, CDC is assembling a detailed clinical picture of the current cases, says Hughes. The goal is to develop an algorithm that physicians can follow when deciding who to monitor closely. "That's on the fast track here," Hughes says.

In contrast to the dearth of diagnostic tools, there's an arsenal of tests to deal with environmental samples, such as suspicious packages and swabs from mail-sorting machines. First on the scene at the Hart Senate Office Building, for instance, were hazardous materials teams wielding hand-held devices smaller than a pack of cigarettes. These devices are a refinement of an instrument first developed in the early 1990s by researchers led by James Burans at the Naval Medical Research Center in Silver Spring, Maryland. The device works on a simple idea: A bit of the suspicious material is suspended in a buffer liquid and allowed to run across a membrane that contains anthrax-specific antibodies, attached to gold particles. If the sample contains the microbes, they'll drag the antibodies with them, creating a visible line somewhere on the membrane within 15 minutes.

Recently this "hand-held assay," as it is known in Navy circles, has been transformed into an easy-to-use commercial test strip—much like a home pregnancy test—by Tetracore, a company founded by former Navy researchers. The test, called BioThreat Alert, is now being sold to cities, counties, hazardous materials teams, and security companies in record numbers. (Tetracore CEO William Nelson says he has even been approached by people interested in turning the assay into a home test kit. "They say they can sell 20 million of these tomorrow if we put them in Wal-Mart," he says—but home testing would be a dangerous idea, he adds.)

Although easy to use, these rapid tests are not very sensitive; BioThreat Alert, for instance, requires some 10,000 spores to give a positive reading, says Nelson—so the test needs to be followed by a more sensitive one. These devices also are not very specific, says Calvin Chue, an expert in pathogen detection at the Johns Hopkins Center for Civilian Biodefense Studies in Baltimore: The antibodies can cross-react with closely related and harmless microbes present in the environment, such as *Bacillus cereus*.

To test environmental swabs from mailrooms and offices, CDC relies on an old standby: a culture test. Environmental sam-

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ples are placed in a broth, allowing the bacteria, if they are present, to multiply. Once grown in sufficient quantities, the bacterial colonies can be tested in several ways. For instance, anthrax colonies growing on a dish with sheep blood agar usually have a "tenacious consistency," according to a CDC lab manual, and when prodded, "the growth will stand up like beaten egg white." With so-called Gram staining, the bacteria often appear as encapsulated, purple rods under a microscope.

Test case. Small and portable PCR machines help detect biowarfare agents even when there are no labs around.

Culture tests are highly sensitive, says Chue, and they rarely yield false positives. But they take 18 to 24 hours to finish. The high sensitivity also poses a new problem: It can detect amounts whose meaning from a public health point of view is unclear. Tests at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) led researchers to conclude that 8000 to 10,000 spores are needed to infect a person. (By comparison, it takes only 10 bacteria to infect someone with the plague.) But evidence from this fall's experience suggests that the number may be lower, and it's hard to say whether any amount is "medically insignificant," as the CIA wishfully labeled the traces found in its mailroom.

Nor does anybody know whether small numbers of spores might have been present in some buildings even before contaminated letters entered the mail system. Very small numbers of spores lie dormant in the soil in many places. Researchers say it seems unlikely that they would also hang around in urban offices, but until last month, nobody had ever looked systematically.

Another highly sensitive test—the so-called polymerase chain reaction (PCR)—can give an answer much more quickly than a culture. In PCR, enzymes seek out and amplify stretches of DNA unique to the microbe and copy them repeatedly until they become detectable—say, with a fluorescently labeled probe molecule. Most PCR machines can produce an answer within 2 to 4 hours.

But PCR is still fairly labor-intensive, and it also has the risk of false positives, because amplified DNA from one sample is easily carried over to another. So for now, says Hughes, CDC is sticking to culture tests for the huge volumes it's processing.

But PCR will assume a more prominent role in the detection of pathogens now that the technology is being miniaturized and made portable, predicts Stephen Morse, a public health professor at Columbia University and a former manager of the Advanced Diagnostics Program at the Defense Advanced Research Projects Agency (DARPA). PCR amplifies genetic material through a series of cycles in which the sample is heated—causing the double helix of DNA to split—and cooled, to let the polymerase enzyme assemble a new copy on each of the two strands.

Companies such as Cepheid in Sunnyvale, California, and Idaho Technology in Salt Lake City, Utah, have built PCR machines whose reaction vessels are minuscule and can be heated and cooled in seconds; a thorough test can be completed in as little as 20 minutes. Just like the videophones that allow modern-day war correspondents to work in the most remote locales, portable versions of these new PCR machines fit in a hefty suitcase that can be taken virtually anywhere. And because the reaction is monitored from the outside, the vessels don't need to be opened at the end, as with older PCR machines, says Cepheid vice president Bill McMillan, limiting the risk of contamination and false positives.

Until recently, CDC and the military bought limited numbers of these machines; now demand has soared, and Cepheid's stock price has tripled since 11 September.

With funding from USAMRIID and DARPA, among others, Cepheid is also trying to eliminate another time-consuming step: sample preparation, which includes steps such as breaking open cells, purifying the DNA, and removing compounds that inhibit polymerase enzymes. The goal is to have all those steps done inside a disposable cartridge. The military's "dream," says McMillan, "is that kids just out of high school can run them." The company plans to present a prototype cartridge for anthrax to the Army by the end of the year.

Beyond PCR

But even such PCR tests probably would not be enough to deal with a large-scale bioterrorist attack or the use of biowarfare agents in a war, says Morse. Tens of thousands of people would need to be tested—which is why the military is funding research into the next generation of diagnostics.

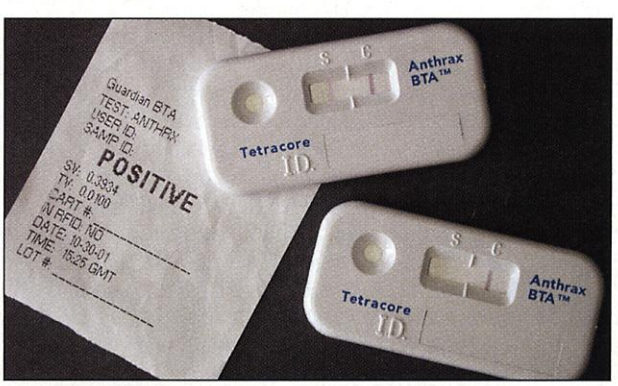
Some researchers are looking at microarrays, or "DNA chips." Fast, small, and accurate, these chips would be ideal for use in the field. Chips can scan for the activity of hundreds or even thousands of genes at once, after which a computer could compare the pattern of active genes with a database of known pathogens and make a quick identification.

In yet another approach, called matrix-assisted laser desorption/ionization mass spectroscopy, microbes' contents are shattered, producing a spectrum of, say, peptides or nucleic acids, which again can be compared to similar spectra of known bacteria.

Future techniques may also overcome the current difficulty of determining who's infected and who's not. A team led by Robert Lad at the University of Maine, Orono, has received DARPA funding to develop and test sensors that could detect minute levels of nitric oxide—a compound released by blood cells called macrophages, the body's scavenger cells, during an infection in the lungs. Such a device could help doctors decide who might have anthrax or another pulmonary infection early in the course of the disease.

However, that project is currently "just conceptual," says Morse—and even if it proves possible, it would take years of research. For now, health officials have to make do with what they have—and people who have potentially been exposed will have to go on taking their antibiotics.

—MARTIN ENSERINK



Are you positive? Hand-held anthrax tests are fast but less accurate.

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