EPIDEMIOLOGY

GERM C

Machines that can quickly identify virtually any bacterium, virus or fungus are being developed for hospitals. Networking the devices could allow health authorities to save lives by spotting disease outbreaks earlier than ever before

By David J. Ecker

Illustration by I Love Dust

ATCHER

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I ONCE WALKED THROUGH AN OLD GRAVEYARD near Philadelphia and noticed the years of births and deaths carved on the headstones. It reminded me that up until the early 1900s, most people died before their 50th birthday. The primary causes of these deaths were infectious diseases such as smallpox, influenza and pneumonia.

Today contagious illnesses kill more rarely in developed nations, where improvements in sanitation, nutrition and vaccines and the introduction of antibiotics have virtually eliminated premature deaths from such afflictions. Yet we are perilously close to returning to an era of untimely deaths from these illnesses because many microorganisms are becoming resistant to existing drugs and because the pharmaceutical industry is not developing enough replacements.

Overprescription of antibiotics is one of the most important contributors to this problem and occurs for understandable reasons. Current diagnostic tools typically cannot quickly determine whether any of a wide range of bacteria—the only organisms susceptible to antibiotics—are making someone sick. In most cases, old-fashioned culturing methods that take several days to complete are required to identify specific bacterial strains. Delaying treatment could prove deadly, and so physicians may try to cover all likely possibilities by prescribing powerful so-called broad-spectrum antibiotics that can dispatch many kinds of germs. At times, however, the drugs kill off susceptible bacteria but leave some that are resistant to that particular medication. These antibiotic-resistant bugs then multiply unchecked by their now absent competitors and can silently spread to other people until finding the right conditions to sicken someone else. Such treatment practices help to safeguard many patients' health today, but they also unavoidably guarantee the emergence of more drug-resistant bacteria tomorrow.

Solutions to this paradox may be at hand. New molecular biosensors are being developed that will allow physicians to quickly determine whether a patient is suffering from a bacterial or other kind of infection and which species is responsible. A key time-saving feature of these devices will be that they look for almost all pathogens at once, instead of testing for individual microorganisms, one at a time. Furthermore, clinicians who suspect that bacteria are at work will not have to guess which species might be present. My research at Ibis Biosciences, which is now a part of Abbott, provides the foundation for one such device. Other bioengineers are racing to develop similar products at other companies.

These rapid diagnostic machines are on track to become commercially available in hospitals and clinics in the next few years. With a little bit of forethought and planning, however, we can greatly magnify their benefit by joining them together in a nationwide or even global network of interconnected devices that would provide the first broad-based, real-time early-warning system for outbreaks of new diseases, foodborne illnesses, global pandemics and, potentially, attacks from bioterrorists.

TIME FOR AN UPGRADE

CURRENT METHODS for diagnosing infectious disease are based on culture techniques that date back more than 150 years to Louis Pasteur. Clinicians collect a sample of a patient's tissue—blood, mucus or urine, for example—and transfer it to a nutrient-rich culture bottle or onto a plate containing agar, a gelatin-like seaweed extract that allows pathogens to grow. After a day or two, the individual microbes will multiply so much that laboratory technicians can identify them. Seeing whether—and how quickly—these cultures fail and die when grown in the presence of various drugs also gives an idea of their sensitivity to different medications. Even if this approach were less time-consuming, however, it would not be ideal for making treatment decisions, because many pathogens—for example, those that need special media or growth environments—can be tricky to culture. Sometimes it is impossible to culture bacteria from patients because they might have already been treated with an antibiotic before the specimen was taken for culture.

I first became interested in the problem of infectious disease diagnosis and tracking while working for the Defense Advanced

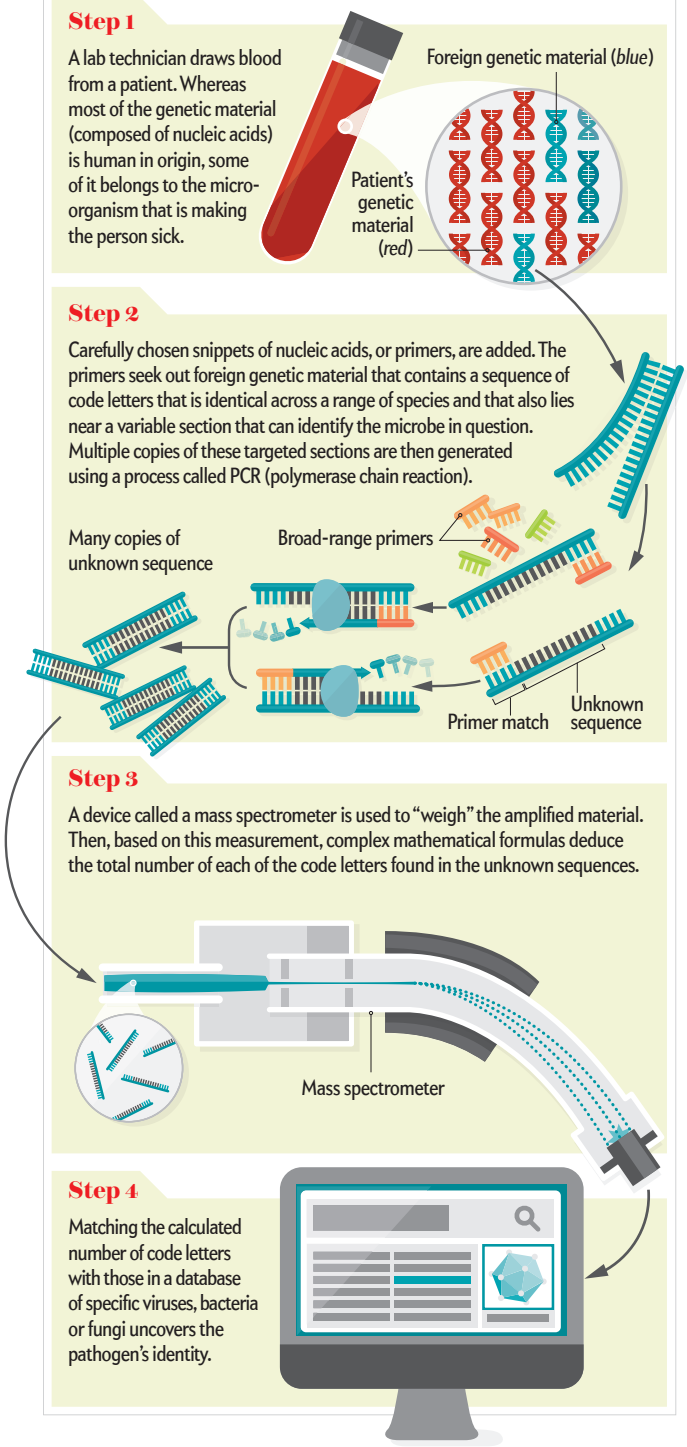
IN BRIEF

New biosensors are being developed that can identify the viral, bacterial or fungal origin of disease or infection within a few hours of testing a sample from a patient.
Individuals would receive the right treatment sooner, and doctors would be more likely to prescribe antibiotics only when they were truly necessary.
Connecting as few as 200 of these biosensors together into a network could offer the U.S. early warnings of emerging epidemics or bioterrorism attacks.
The greatest obstacles to creating such a network are mostly political and regulatory challenges—not technical ones.

HOW IT WORKS

Instant Biosensor

Sophisticated devices that employ a combination of biological, physical and mathematical tools (*shown below*) are being developed that can identify any of more than 1,000 pathogens that cause human illness. A network of such biosensors distributed across a country or region could provide early warnings of disease outbreaks or biological attacks and identify the most effective treatments.



Research Projects Agency on new approaches to discovering antibiotics. Our goal was to pick through thousands of compounds to find a few that disabled many different kinds of bacteria by gumming up a specific stretch of RNA—a molecule that is central to the machinery of all living cells—that they shared.

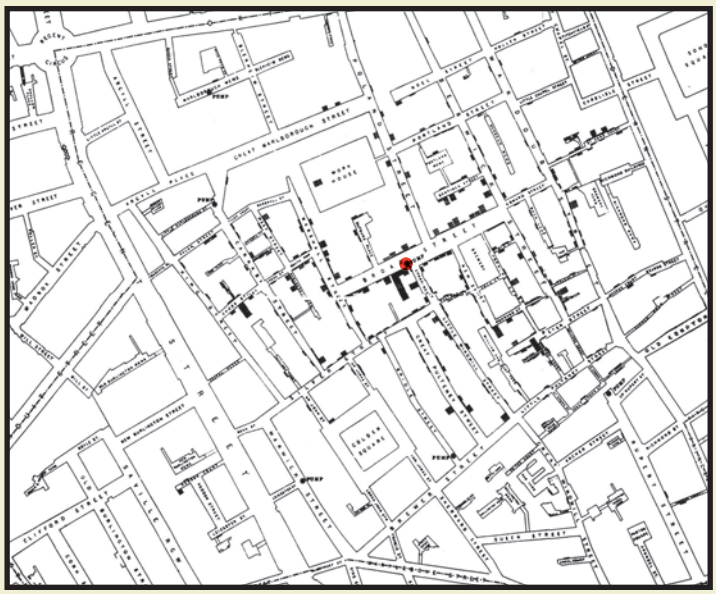
My colleagues and I used devices called mass spectrometers to determine whether the potential drugs had attached themselves to the bacterial RNA. Mass spectrometers are essentially scales that weigh molecules very accurately. (Formally speaking, they determine their mass.) Because we knew the weight of the bacterial RNA in question, we could deduce the weight of any compound that was stuck to it in the same way you might weigh a dog by holding it while standing on a bathroom scale and then subtracting your own weight. Knowing the compound's weight in turn gave us its identity because each compound had its own unique mass.

We soon realized that this same technology could allow us to distinguish among bacteria, viruses, fungi and parasites by weighing some of the organism's RNA or DNA, which is a closely related type of nucleic acid. Each strand of these molecules is made up of subunits, or nucleotides, that are often referred to by the letters that distinguish their nitrogen-containing sections—A (adenine), C (cytosine), G (guanine) and either U (uracil), in the case of RNA, or T (thymine), in the case of DNA. Practically by definition, some portion of those nucleic acids will be unique to different pathogens. Because the molecular weights of the various nucleotides (A, T, C, G and U) significantly differ from one another, we can determine the numbers of each nucleotide present in a particular strand based solely on its mass spectrometry reading. For example, any DNA strand that weighs 38,765.05 daltons (daltons are the standard unit of atomic measurement), as determined by a mass spectrometer, must contain 43 adenine, 28 guanine, 19 cytosine and 35 thymine subunits; that combination is the only one to give precisely that result without having to resort to fractions of a nucleotide, which do not exist in nature. And that information, in turn, tells you what species of microorganism is present.

The method is similar to one you might use to calculate the numbers of unblemished coins in a jar containing only U.S. quarters (which weigh 5.670 grams apiece when brand-new) and nickels (which weigh 5.000 grams apiece). If the total collection weighs 64.69 grams, it must consist of seven quarters and five nickels ($64.69 = 5.67q + 5n$, where q and n can only be positive integers or zero). Any other number of quarters would require fractional nickels.

The process for identifying pathogens requires an ability to distinguish the culprit's DNA or RNA from the patient's own in a specimen being tested. Usually the amount of foreign material is too scant to allow for meaningful measurements unless additional copies are made. Instead of having to wait until more microbes can be grown in culture, however, we use a technique called PCR (for polymerase chain reaction, named after enzymes that can duplicate nucleic acids) to make copies—or amplify—the DNA or RNA present in a sample from a patient. PCR has been used for a long time to detect pathogens, but it has been limited to detecting one or just a few pathogens at a time. My colleagues and I decided to use PCR in a way that, when coupled with mass spectrometry, enables detection of very broad groups of organisms at the same time.

Illustration by Lucy Reading-Ikkanda



MAPPING OF CHOLERA CASES (*black shading*) **in 1854 London led a physician named John Snow to the source of the illness: a public pump delivering water contaminated by feces (*added red mark*). Similar mapping is done today to pinpoint the origin of disease outbreaks and bioterror attacks. The author's proposed network of pathogen sensors would identify the sources of outbreaks much more quickly and easily than is currently possible.**

The key element is limiting the amount of nucleic acid we have to generate to get definitive results. We achieve that aim by being very picky about the segments of DNA or RNA that we amplify. We make sure to select fragments that contain sequences of letters that are identical, or conserved, across broad groups of organisms—such as all organisms that can be tinted with a so-called Gram stain versus those that cannot—and that are adjacent to regions unique to a particular species such as *Staphylococcus aureus*. Targeting multiple carefully chosen sequences allows us to identify precise categories and subcategories of organisms without having to lengthen the process unnecessarily. So after extracting the microorganism's RNA or DNA, we add primers (short pieces of DNA that mimic a living cell's natural mechanism to initiate the process of copying nucleotides) to the sample, which will then pick out the desired segments for further processing. After that process is complete, we measure the segments in the mass spectrometer, which gives us a distinct series of numbers that we can cross-reference to a master database we have collected of the more than 1,000 organisms known to cause disease in humans.

Together the hardware and software constitute a universal pathogen detector capable of identifying the type of organism responsible for a person's illness, as well as some of its unique identifying characteristics, in just a few hours.

A prototype of the machine that I helped to create was put to the test under real-world conditions in 2009, when a nine-year-old girl and a 10-year-old boy with flulike symptoms showed up at two different locations in southern California. Clinicians swabbed the children's throats and subjected the samples to standard rapid screening tests for flu. The results suggested that influenza virus was responsible for the youngsters' illnesses but were unable to say which of the then known strains of virus was responsible.

The samples were sent to the nearby Naval Health Research Center in San Diego, which was running numerous tests of the prototype device. The instrument correctly determined that the two children had been infected with the same viral strain, which had never been seen before. The device also pinpointed the virus's recent origin in pigs because the count of the RNA letters most closely matched strains of influenza from pigs in the database. Furthermore, the mass spectrometer's count of the letters, or fingerprint, of the two first cases matched later samples of what was soon referred to as the swine flu virus—now known as pandemic (H1N1) 2009 virus. No one can say whether the early warning saved any lives, but it certainly did not hurt, and having technology in routine use that called out a strain of virus as new and unique would undoubtedly have value for identifying new outbreaks.

As important as it was to quickly identify the new flu virus in 2009, universal pathogen detectors are expected to really shine in situations where clinicians have no clue what is making their patients ill. The devices can also help when selecting drugs. Notably the same mass spectrometric profile that reveals the strain of a bacterium provides clues to its susceptibility to various antibiotics, allowing doctors to prescribe the correct antibiotics right away and only when they are truly needed. Patients should benefit from faster recovery time, even for resistant strains, because they receive the optimal therapy sooner.

BEYOND SINGLE PATIENTS

MOVING FROM INDIVIDUAL to societal benefits, clinicians will quickly be able to determine if several people in one area have been infected by the same organism—for example, *Salmonella,* which is a common cause of food poisoning. You might expect that once public health investigators have such information, they would conduct an old-fashioned, shoe-leather epidemiological investigation, interviewing patients and tracing their recent movements to determine whether they all have something in common—such as having been a patron at a particular restaurant or eating the same specific salad ingredient. The results from such investigations, which follow the same basic format as John Snow used in 1854 to trace the cause of a cholera epidemic in London to a shared water pump, can take weeks to months to complete—which explains why only the most severe outbreaks are usually investigated or solved.

There is a better way, however—and the key to achieving it is probably sitting in your pocket or purse right now. Most people today carry a mobile phone, which maintains geolocation data as part of the operating software or in one of the ancillary applications. In addition, service providers collect all types of cell-tower information that can triangulate a person's whereabouts at any

JOHN SNOW, 1854

given time. If patients infected with an organism of public health importance were to volunteer to share their recent travel history from their cell phone, epidemiologists could rapidly determine whether several patients sickened with the same organisms had visited the same location within a specific time window.

The same right to privacy that must be respected in current epidemiological investigations would need to be preserved in a cell-based system as well. The biggest difference: the answers would come a lot faster. Properly coordinated, the data from a well-designed network of universal pathogen detectors would do more than allow essentially instantaneous identification of such public health threats as an epidemic outbreak, a bioterror attack or a potentially life-threatening contamination of the food supply. In addition, public health experts would know right away where an infection might have originated and whether the event was contained to a single city or had already spread to multiple cities. Results could be quickly reported to individual patients or health authorities as needed, and doctors could expedite sharing information about effective treatments.

Building such a network—I call it "Threat Net"—would finally bring medical diagnostics and epidemiology out of the 19th century and squarely into the 21st.

HOW BIG A NETWORK?

BECAUSE THE SPREAD of infection can be represented as a social network, we can determine mathematically how many pathogen detectors should be connected to one another for the entire enterprise to function as an effective early-warning system across a country or region. One of the easiest ways to approach the problem is to use a mathematical model called a Monte Carlo simulation, in which a computer runs the same scenario under multiple conditions to determine a range of probable outcomes. (Investment firms use similar calculations all the time to estimate the size of a person's nest egg at retirement, under several potential market conditions.) Given known national epidemiological data on infection rates, where and how symptomatic people seek health care, how often diagnostic tests are ordered and the incubation times of a wide range of infecting organisms, I ran the numbers thousands of times to determine the size at which the network would begin providing an early alert about a national outbreak of a public health–relevant organism.

The results were remarkable. Linking 200 carefully chosen hospitals across the nation to the network would be sufficient to cover the entire U.S. metropolitan population. Each urban area the size of Washington, D.C., or San Diego would need about five hospitals with universal biosensors on the network; there would be a 95 percent probability of immediately detecting a public health–relevant infectious agent, such as bird flu, anthrax, plague or a foodborne pathogen if only seven patients sought care in an emergency department.

This unexpectedly low number of networked machines, or nodes, is driven by a phenomenon that I refer to as "the funnel effect." Most sick people stay home to nurse themselves. But the sickest individuals will manage to get themselves to a hospital (the first funnel), where trained physicians (the second funnel) will decide which of them needs to be tested. In other words, we do not have to put biosensors where the people are—which would require more devices; enough of the "right" people will bring, or funnel, themselves to the biosensors.

When I conducted computer simulations of the most common public health–relevant infectious diseases and compared the performance of Threat Net in identifying new outbreaks with the best possible performance of the current system, Threat Net was far superior. It identified the leading edge of the outbreak, several days to weeks before the current system. In a real-world context, having even a few days' advance notice of an outbreak could mean the difference between life and death for thousands of people, as hospitals prepare for an influx of patients, health authorities release stockpiles of medications or investigators determine the source of a malevolent attack.

WHAT'S NEXT?

BY MY CALCULATIONS, it would cost about $40 million to establish a network of 200 hospitals (assuming the hospitals buy their own biosensors) and then about $15 million a year to maintain the network. In contrast, a 2012 study of the 14 most common causes of severe foodborne illness put the direct costs of treatment and missed work at $14 billion a year. In the U.S., it would probably make the most sense for the Centers for Disease Control and Prevention to run the network—given its current expertise and mission for tracking outbreaks.

No one has ever developed an epidemiological surveillance system as sophisticated as Threat Net. Given past experience, designing the hardware and software will probably be the easiest part. Many regulatory, legal and turf issues must also be addressed. But the greatest obstacle is that no single stakeholder has the mandate, incentive or opportunity to launch such an undertaking—even though everybody's global interest would be served. The level of cooperation needed from physicians, nurses, hospital administrators, public health experts and privacy advocates may be especially hard to achieve in countries with decentralized and mostly private health care systems.

A society-wide integrated approach to infectious disease diagnosis will be more effective and substantially less expensive than the current approach to public health and medical countermeasures for the detection of pandemic agents and biothreats. The concept of piggybacking real-time public health surveillance onto next-generation diagnostic technology, in combination with modern network and communications technology, has great potential to improve patient care, spare antimicrobial use, and provide alerts that would enable earlier containment of outbreaks or bioterror attacks. What remains to be seen is whether we are wise enough to combine our efforts to produce a smarter health surveillance system. SA

MORE TO EXPLORE

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FROM THE ARCHIVES

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