EMERGING DISEASES:
NEW and OLD
Take a Role…

In the early 1980s, it took almost three years to identify the cause of AIDS. In contrast, scientists using biotechnology made it possible to identify the viral agent causing SARS (sudden acute respiratory syndrome) in eight days. Its genome was sequenced in six days, paving the way for efforts to develop treatments and vaccines.

The ability to determine the structure of microorganisms, sequence their entire genetic material (genome), and figure out how the genes and the proteins they control work (proteomics) gives us tools that we’ve never had before in our continuing struggle with emerging diseases, old and new. Using computers to help us predict the behavior of an illness is also another way that technology and biology combine.

Infectious disease is a vital, exciting area of research. We hope these articles get you thinking about how you could help make the world a safer place through a career in biotechnology.

Paul A. Hanle
President
Biotechnology Institute

FUN FACT Some microorganisms cause harm; most don’t. You have about 2 million bacteria living on your face. About half of your feces is made up of useful bacteria that have been living in your gut.
Coming Soon to a Person Near You

In a dark theater, you watch as a monster emerges. It begins killing people and making the rest hysterical. And just when the hero thinks the monster is dead, it springs out in some shriek-producing way. In the health world, that monster could be infectious disease.

SARS and mad cow disease are the latest new diseases in North American headlines. They join such illnesses as Lyme disease (1975); Legionnaires’ disease, a severe form of pneumonia (1976); and AIDS (1983). Along with these emerging diseases come reemerging ones, like monsters reawakening. For example, polio has been spreading in Africa after one country suspended vaccinations briefly, and malaria has been reported in Virginia.

Around the world, medical personnel are running up against new forms of familiar diseases that can’t be prevented by vaccines or treated with drugs. Madeline Drexler, author of Secret Agents, discusses these “super-bugs” (p. 10). When it comes to adapting, viruses, bacteria, fungi, and protozoa—called “microorganisms”—are way ahead of humans. A generation for us is 20 years. For a bacterium, it’s every 20 or 30 minutes. Even more alarming is that it takes, on average, 17 years to come up with an antibiotic to fight a disease-carrying bacterium that can develop resistance practically as fast as it replicates.

And, as if all that wasn’t enough, the threat of biological warfare looms, dredging up fearsome illnesses. Smallpox, anthrax, plague, botulism, tularemia, and viral hemorrhagic fevers such as the Ebola virus are considered by the Centers for Disease Control and Prevention to be the likeliest agents of bioterrorism.

In the industrialized world, we’ve come to rely on vaccines and antibiotics to protect us from disease. Our known monsters seemed taken care of. But with the ease and speed of international travel, it is more likely that no one will be safe from diseases that are widespread in less developed countries, such as Chagas disease, dengue fever, rotavirus, and leishmaniasis.

Public health officials agree that research for a vaccine or treatment ramps up significantly when an illness begins hitting North Americans. West Nile virus has been circulating in other parts of the world for decades, but now that it’s in the United States, a vaccine for horses is already on the market and a vaccine for humans is in clinical trials—the last step before it can be approved by the Food and Drug Administration.

Fighting new and old infectious diseases demands the attention of many disciplines. Government researchers have to work with those at universities, public organizations, and private companies to find ways to identify and counter diseases.

Such a multidisciplinary approach is helping, for example, in the global fight on tuberculosis. A private biotech company funded by the U.S. National Institutes of Health has developed the first tuberculosis vaccine to be tested in people in the United States in more than 60 years. Using recombinant DNA technology, the vaccine contains two fused TB proteins combined with substances that further boost the immune system’s response (adjuvants).

Biotechnology plays a part in the response to any new disease. In this issue we focus on human health, but animals and plants are also affected. West Nile virus rarely causes death in people, but about three out of every 10 infected horses die. Plant diseases caused by viruses, fungi, bacteria, and phytoplasmas are a major constraint on crop production.

Using biotechnology can help scientists quickly detect and diagnose diseases. At the same time, biotechnology joins basic research in helping develop medical countermeasures, such as surveillance tools, diagnostic tests, vaccines, and treatments.

Read on to learn about vaccines, the immune system, vectors, the mathematics of epidemics, ethical responses to infectious diseases, and how bug spit can help spot emerging diseases.

—Lois M. Baron
Emerging Diseases: New and Old

Infectious diseases are caused by microbes—organisms too small to be seen without a microscope. Many microbes, such as bacteria, are made up of only one cell. Viruses, mere snippets of genetic material packed inside a membrane or a protein shell, are even smaller. Humans evolved an immune system because the world is teeming with these organisms. Many of them don’t bother us; the bacteria that normally live in your gut are, in fact, beneficial. But some microbes can do you great harm.

Your immune system is a complex network of cells and organs that evolved to fight off infectious microbes. Much of the immune system’s work is carried out by an army of various specialized cells, each type designed to fight disease in a particular way. An invading microbe first runs into big, tough, patrolling white blood cells called macrophages (literally, “big eaters”). The macrophages grab onto and gobble up as many of the microbes as they can, engulfing them into their blob-like bodies.

How do macrophages recognize microbes? All cells and microbes wear a “uniform” made up of molecules that cover their surfaces. The uniform contains marker molecules unique to you and only you. By “feeling” for these markers, the macrophages and other cells of your immune system can distinguish among the cells that are part of your body and harmful invading microbes that need to be destroyed. The microbe molecules that identify it as foreign and stimulate the immune system to attack it are called antigens. Every microbe carries its own unique set of antigens.

Macrophages behave like Paul Revere by carrying the message of microbe invasion to the immune system’s base camps, also known as lymph nodes. The message consists of both antigens and digested fragments of invading microbes. The messenger macrophages stimulate production of immune system soldiers, B cells and T cells, specifically trained to fight the invasion.

T cells function either offensively or defensively. Offensive T cells don’t attack a microbe, but instead they eliminate the cells of your body that are infected. Because they have been “programmed” by their exposure to the virus antigen, these cytotoxic T cells, also called killer T cells, can “sense” diseased cells infected by the microbe, latch onto them and release chemicals that destroy both the cells and the microbes inside. Defensive T cells, also called helper T cells, assist in activating killer T cells and B cells.

B cells are like weapons factories. They secrete extremely important molecular weapons called antibodies. Antibody molecules combine with antigen molecules like the pieces of a jigsaw puzzle fit together—if their shapes are compatible, they stick together. Each B cell can make only a single antibody and each antibody can stick to only one antigen. So your immune system keeps a supply of millions and possibly billions of different B cells making different antibodies on hand to be prepared for any foreign invader. The messenger macrophage and helper T cells recruit the right B cell soldiers to replicate making more of the needed weapon factories to produce antibodies.

On average, your immune system takes more than a week to learn how to fight off an unfamiliar microbe. Sometimes that isn’t soon enough. Stronger microbes can spread through your body faster than the immune system can fend them off. Your body often gains the upper hand after a few weeks, but in
You’re more likely to pick up disease-causing microorganisms from an ATM or a handshake than from a public bathroom doorknob, says a recent study. The best everyday defense against disease is to wash your hands—a lot. <newwise.com/articles/view/505673/>

### Vaccine Types
<table>
<thead>
<tr>
<th>Vaccine Types</th>
<th>How Made</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Live, attenuated vaccine</td>
<td>Virus is weakened and used.</td>
<td>Produces a strong immune response; often gives lifelong immunity.</td>
<td>Remote chance that microbe could revert to virulent form.</td>
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<tr>
<td>Inactivated or “killed” vaccine</td>
<td>Virus inactivated with chemicals.</td>
<td>Safer, more stable; easily stored and moved; no refrigeration required.</td>
<td>A weaker immunity than with live vaccines; usually needs booster doses.</td>
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<tr>
<td>Toxoid vaccine</td>
<td>Treats the toxins (poisons) produced by germs with heat or chemicals.</td>
<td>Teaches immune system to fight off bacterial toxins.</td>
<td></td>
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<tr>
<td>Subunit vaccine</td>
<td>Uses antigenic fragments from microbe or made in the lab.</td>
<td>Very specifically targeted; fewer side effects.</td>
<td>Identifying the best antigens for a new vaccine can be difficult and time-consuming.</td>
</tr>
<tr>
<td>Conjugate vaccine</td>
<td>Links proteins or toxins from a type of organism that an immature immune system can recognize to the outer shell of the disease-causing bacteria.</td>
<td>Allows infant immune systems to recognize certain bacteria.</td>
<td></td>
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<tr>
<td>DNA vaccine</td>
<td>Places a few proteins from the DNA of a disease-causing organism into the body’s own cells.</td>
<td>Produces strong, long-lasting immunity. Should be safe because they exclude the genes critical to the pathogen’s survival.</td>
<td></td>
</tr>
<tr>
<td>Recombinant vector vaccine</td>
<td>Harmless genetic material from a pathogen is inserted into a weakened virus or bacterium, which carries it into the host.</td>
<td>Closely mimics a natural infection, prompting a strong immune response.</td>
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Your body “remembers” how to protect itself from the microbes it has encountered before. The previously activated B and T cells behave like reserve military units, ready to be called rapidly into action. Vaccines take advantage of your body’s natural ability to learn how to eliminate almost any disease-causing microbe that attacks it.

Traditional vaccines contain either parts of microbes or whole microbes that have been killed or weakened so that they don’t cause disease. When your immune system confronts these harmless versions of microbes, it quickly clears them from your body. In other words, vaccines teach your body important lessons about how to defeat its opponents. Once your immune system is trained to resist a disease, you are said to be immune to it. Before vaccines, the only way to become immune to a disease was to actually get it and, with luck, survive it. Vaccines provide an easier and less risky way to become immune.

Whether administered through a shot, a liquid or a nasal spray, vaccines give our immune systems a boost to ward off disease.

*Antibodies recognize foreign antigens and mark them for destruction. An epitope is the part of a foreign organism (or its proteins) targeted by antibodies, cytotoxic T cells, or both. Epitopes can be many different shapes.*
The Conundrum of the Killer Coronavirus

How did you know the victims, Mr. SARS? Were you close?

Well, it seems the killer needs to be in close proximity to be passed from one person to the next.

Am I a suspect, detective?

Look, don’t be cute. We know it was you.

Police Headquarters, Interrogation Room, SARS Investigation, Interview with Mr. I.M. SARS.

Isn’t this you at the International Convention of Emerging Viruses talking to the Plague?

You have been found in the lungs of all of the victims!

Those deaths occurred well after my levels peaked.

Their lungs were destroyed by an overreaction of their immune systems.

Maybe you should be talking to him instead of harassing me.

That doesn’t make me a killer.

We also found this in your apartment.

“How to Start an Outbreak” written by Avian Flu.

She’s a wonderful writer.

Tip #2 was “Start at a Chinese open market.”

Ugh, those are such messy places...
you really should shut them down, y’know.

oh, wait, you can’t.

it would be harder for you to monitor problems and outbreaks if the markets went underground wouldn’t it?

tsak what a dreadful situation...

... for you.

all this talk of animals is making me feel left out.

avian flu had birds and the plague had fleas.

but my vector remains... unclear.

we know that’s where you got started.

one third of all the people you’ve infected in china were animal handlers.

we’ll find it, smart guy.

his bond has been posted, detective.

get him outta my sight.

who would bail out that scumbag?

it was a cute little critter. a civet, I think it was. ...the vector...

stop him!

I’ll get you yet, sars!

you just might, detective, if you’re not careful!

never the end...

copyright Jay Hosler
When you’re doing math, do you ever think that lives could be at stake? Probably not (unless your mom gets really frustrated about that unfinished homework …).

When David Fisman was 15, he didn’t think math was important either. But as a young doctor in Ontario, Canada, in March 2003, he learned math and computers can save lives—by helping us understand how diseases spread and how to control epidemics.

David was working in a public health department in Hamilton, Ontario, when the sudden acute respiratory syndrome (SARS) broke out in Toronto, about 40 miles to the east. Because David is an epidemiologist, the branch of medicine that studies the spread of diseases, he was tapped to help Toronto’s public health officials figure out how to contain SARS.

As a volunteer on an emergency committee, David thought he might have been exposed to the SARS virus by a fellow committee member, a doctor who had treated SARS patients. To avoid infecting other people, David worked at home for a week and waited to see if he would get sick. That gave him lots of time alone to work on a simple computer model predicting how SARS might spread and how many people might end up being infected.

### A Simple Idea

The basic idea behind mathematical models of diseases like SARS is not new. People have used math to predict how diseases will behave for more than 200 years. Mathematical models reduce the real world’s messiness and complexity to a few simple equations.

Virtually all mathematical models of disease divide people into groups: susceptible (able to catch the disease), exposed (to the disease), infectious (able to spread the disease to others), and immune (recovered from or vaccinated against the disease). Models use mathematical equations and medical statistics to track the people as they move from group to group.

With a new disease, like SARS, the entire population is in the “susceptible” group at first. Then some individuals are “exposed” to the disease and may become “infectious” for a given time (usually a few days). During this period, they cause more people to move from the susceptible to the exposed and infectious groups. Following this infectious period, people move into the “immune” group. As more people enter the immune group, fewer people are left in the susceptible group, and the number of new disease cases declines. Because of this decline, not everyone in the susceptible group is infected before the disease dies out.

In the real world, the picture is not so simple. Epidemiologists sometimes create detailed, multilayered computer models to represent complex diseases that spread differently among different groups of people, such as diseases that affect mainly children or intravenous drug users. However, even the simplest model can provide researchers with important insights about the likely course of an epidemic. For example, computer modeling of the SARS outbreak in Vietnam showed that if control measures had been delayed one month after the first case, instead of only a week after, there would have been 1,000 cases—instead of 62.
Fear into Hope

As David Fisman worked on his mathematical model, at first he was alarmed. The number of SARS cases shot up quickly in the first two weeks of the Canadian outbreak, and no one knew whether the disease could be controlled before thousands of people got sick. But one thing that mathematical models can predict is how well disease control measures like quarantines will work.

Quarantine is an old idea—in the 1300s, ships had to anchor off Venice for 40 days to make sure they were free of bubonic plague before anyone could come ashore. In Canada, about 10,000 people exposed to SARS were quarantined at home for 10 days to see if they would get sick. This policy was unpopular, because people without symptoms did not appreciate missing work and not seeing their families and friends. David’s simple model and the more complex version developed by his former professor at Harvard showed that measures like isolating people with SARS symptoms in the hospital and quarantining people exposed to SARS could stop the epidemic. This information can give people a reason to stick with inconvenient but necessary policies like quarantines.

Thankfully, David didn’t get sick. He reported back to work to help deal with the SARS health-care crisis. He is now an assistant professor of epidemiology and biostatistics at Drexel University in Philadelphia.

“I wish someone had convinced me at 15 that math could actually be useful,” he says. “If they had, I probably would have had less catching up to do as an epidemiologist!”

–Karen Holmes

Fun Fact
There are more than 100 common cold viruses.

DID YOU KNOW?
In Hong Kong about 300 people at an apartment complex were exposed to SARS by a single virus-laden visitor. Investigators studying the outbreak came up with some unusual conclusions. They used detailed epidemiological studies and sophisticated computer modeling of airflows to show that faulty engineering could have caused a toilet used by the visitor to contain the virus in aerosol particles (which every toilet puts out). These particles were sucked into the ventilation system, drawn upward in a contaminated air plume, and spread within and between buildings by air currents. –K.H.
Pimples are one of the mild infections caused by *Staphylococcus aureus* bacteria. But staph, as it’s often called, can cause serious and sometimes deadly infections that aren’t fazed by the medicines of first choice, antibiotics. These infections are caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Hospital patients are at risk, but more infections are being reported among otherwise healthy people.

In February 2003, the U.S. Centers for Disease Control and Prevention reported a frightening staph epidemic in Los Angeles County. Three separate outbreaks had struck among mostly healthy people, including school athletes on the same team. In the late ’90s, a different MRSA strain had killed four children in North Dakota and Minnesota and sickened scores of others.

Such outbreaks trigger alarm because they signal that antibiotics—the greatest lifesaving drugs in history—may be losing their effectiveness. If antibiotic resistance increases, we could return to an age where daily life would be an obstacle course of fear.

Before antibiotics were discovered in the early 20th century, a staph infection that entered the bloodstream killed 90 percent of its victims. A man who nicked himself shaving could die from erysipelas, a strep infection. Children often lost playmates to scarlet fever, meningitis, and osteomyelitis.

Antibiotics are drugs that kill bacteria, one-celled organisms that are usually visible under a microscope. Though not all bacteria cause disease, they’re responsible for many serious diseases—such as tuberculosis, common forms of pneumonia, children’s ear infections, and many foodborne illnesses.

In 1928, Scottish bacteriologist Alexander Fleming discovered the first major “miracle drug,” penicillin. Since then, other classes of antibiotics have been developed, such as tetracyclines, macrolides, and fluoroquinolones. Each class works in a somewhat different way and is often aimed at a different group of pathogens.

Unfortunately, genetic mutations enable pathogens to “adapt” to an antibiotic. For example, in a group of presumably identical pathogens, each pathogen differs slightly from the next by subtle mutations, DNA or RNA copying errors that occur during replication. Some
mutations give bacteria altered properties, usually deleterious ones. When an antibiotic attacks a group of bacteria, those susceptible to the drug die. Mutant bacteria resistant to the drug can survive and multiply. The altered genes can become part of a mobile genetic element called a transposon that lets bacteria share bits of their genetic information with other bacteria. And the more the drug-resistant transposons spread, the more they add to the pool of resistance genes in other bacterial strains.

These adaptations have led to a rising tide of antibiotic resistance worldwide. In poor nations, resistance has sprung up in bacteria that cause infections ranging from tuberculosis to dysentery; in wealthier nations, drug resistance has been seen in bacteria that cause diseases ranging from foodborne Salmonella infections to sexually transmitted gonorrhea.

How do we solve antibiotic resistance?

Produce new drugs faster. Though today’s antibiotics are mostly derived from nature, tomorrow’s may be plucked from huge collections of man-made molecules generated from knowledge of the genome that, with new technology, can be tested in large numbers against resistant organisms. Gene sequencing will speed up this process by revealing ways to disarm bacteria.

Revamp antibiotics that have lost their clout. For instance, at least one laboratory is creating new tetracyclines that can interfere with the genetic “master switch” used by bacteria such as Salmonella and E. coli to resist antibiotics.

Explore the use of bacteriophages—tiny viruses that target bacteria. Today, several biotech companies are trying to grow phages that fight MRSA and other pathogens.

Develop vaccines that protect against bacterial diseases. Vaccines stimulate protective immune responses that kill and clear the bacteria away before antibiotic-resistant strains are produced.

Antibiotic resistance will never disappear—because bacteria keep changing to outwit humankind’s medical weapons. But prudent use of current antibiotics, coupled with creative biotechnology, can make a potentially catastrophic threat a controllable one.

Did You Know . . . ?

More than 70 percent of the bacteria that cause hospital-acquired infections resist at least one of the drugs most commonly used to treat them.

—Madeline Drexler
Nearly 300 years ago, Edward Jenner observed that milkmaids who had had cowpox did not get sick with smallpox. When he wanted to test his theory of vaccination in 1796, he simply injected his gardener’s eight-year-old son with pus from a cowpox pustule, exposed him to smallpox, and waited to see what happened.

Fortunately, the vaccine worked and did so without nasty side effects. Jenner’s invention became the basis for the smallpox vaccine.

Looking into Disease

For researchers, one huge issue is ensuring that research participants really know what they’re getting into. Getting this “informed consent” means making participants understand that experimental interventions may not help them. In instances where no treatment is currently available, they may even be receiving sugar pills or other placebos.

Misunderstandings can be deadly. People receiving experimental vaccines against HIV/AIDS, for example, may assume they’re protected and stop protecting themselves against infection.

Researchers must also submit their proposals to “institutional review boards.” These experts review proposals and decide whether they’re ethically acceptable.

These and other safeguards arose from the infamous Tuskegee Syphilis Study, a 1932–1972 government project. Hoping to learn more about syphilis, researchers withheld treatment from poor African-American men—without telling them they had the disease that was being studied. The men knew they were sick, but thought they were being treated for “bad blood.” Today, trial participants must, at minimum, receive treatment for their illness; the experimental drug or treatment is measured against standard treatments.

Doing research in poorer countries brings special challenges. Getting informed consent can be extra-challenging in environments where people are unfamiliar with research. Some critics charge that doing research in the developing world is inherently exploitive, especially when using placebos.

Researchers guard against exploitation by doing research that is useful to developing nations, studying HIV/AIDS and other killers. And they try to give something back, such as building clinics, offering scholarships to public health programs, or providing access to vaccines or medications if the research proves successful.
Responding to outbreaks brings different ethical challenges. When a disease like SARS strikes, public health authorities can compel sick people to stay put at home or hospitals to protect others. They use the term “isolation” when restricting the movement of people who are already sick and “quarantine” for people who’ve been exposed but aren’t yet ill (see page 9). In this case, societal protection trumps individual freedom.

Authorities can also force people to use medication or vaccines. People with tuberculosis, for example, can be locked up if they won’t take medication since their actions endanger society.

In dire situations, ethical thresholds may shift. The risk of serious side effects from a vaccine for smallpox—a disease eradicated in nature in 1977—may seem less important in the face of bioterrorism.

In everyday life, some people refuse vaccinations. Some have weakened immune systems. Others object for religious reasons or view enforced vaccinations as an intrusion of privacy. Still others don’t believe the benefits outweigh the risks.

Some parents, for instance, reject childhood immunizations for fear of potential risks that studies indicate as either very small or unproven. The result has been outbreaks of illnesses like measles and whooping cough—and anger from public health specialists who argue that these parents want everyone else to accept risks they won’t accept themselves.

Thanks to a concept called “herd immunity,” however, not every single person has to be vaccinated to protect the group as a whole. If almost everyone is vaccinated, an isolated case of disease simply has nowhere to go. That’s a shot in the arm for all of us!

In 1954, elementary students lined up for shots of the live vaccine against polio. A rumor quickly spread among the children that getting the shot would give you polio. A “Polio Pioneer” received this card for taking part in the test.

When a single case of smallpox popped up in Yugoslavia in 1972, the government quickly quarantined the entire country and vaccinated all 20 million Yugoslavians.

Could the United States be as effective should bioterrorism strike? To find out, a senior-level 2001 exercise called Dark Winter had officials and journalists role-play reactions to an imaginary smallpox outbreak.

Huge ethical questions emerged: When supplies are scarce, whom should receive vaccines? Should the government offer vaccines of unknown safety? Use force to keep the sick isolated? Outlaw travel? The answers weren’t just academic. By the fourth round of infections, a million Americans could have been “dead.”

–R.C.

Is it ethical to be the one who doesn’t get immunized—bypassing the minimal risks involved—but then benefits from “herd immunity” of others who are vaccinated?

–Rebecca A. Clay
When José Ribeiro sees a mosquito land on his arm, his first instinct isn’t to slap it out of existence but to think about how it—and its blood-sucking brethren—deserves a Nobel Prize. What amazes Ribeiro about mosquitoes, sand flies, and other insects that feed on blood is the way they’ve made mealtime easier for themselves. “Getting blood from a mammal isn’t easy,” he explains, noting that multiple mechanisms like clotting and vasoconstriction (constricting blood vessels) work together to keep your blood where it belongs. To break through those defenses and do so without being noticed, some insects inject chemicals into their victims that widen blood vessels to increase blood supplies to the bite site. Others inject anticoagulants to keep blood flowing or substances that keep platelets from clumping together. Some even inject chemicals that act as painkillers. Ribeiro, an insect saliva specialist, is determined to uncover all their secrets.

“To disarm a redundant system, you cannot use a magic bullet,” Ribeiro explains. “You have to use a magic potion. And each insect has invented its own.” His hope is that by deciphering these potions, he’ll discover compounds that could form the basis of medications for bleeding or clotting disorders and other conditions and of vaccines for bug-borne diseases.

A native of a steel-milling city in Brazil, Ribeiro didn’t grow up dreaming of bug spit. In fact, he started out as a physician. But even before he finished med school in 1974, he knew he wanted to be a scientist rather than see patients. He went on to earn a master’s degree and then a doctorate in biophysics in 1981. As a junior faculty member at the Biophysics Institute of the Federal University of Rio de Janeiro, however, he encountered a charismatic entomologist and was soon sucked into the world of insects.

Ribeiro began studying the salivary glands of kissing bugs—which carry the potentially deadly Chagas disease common to Latin America—and thereby became history’s first bug spit specialist. After leaving Brazil, he landed at the University of Arizona in Tucson and then the Harvard School of Public Health. He came to the National Institutes of Health in 1996, which is one of about a dozen institutions studying insect saliva.

As you can imagine, harvesting bug spit is not an easy task. Let’s just say that the insect and its salivary glands or mouthparts are separated. Ribeiro then creates what he half-jokingly calls a “spitome”—the genetic record of the substances in their saliva. After identifying the many separate compounds in the spit, he and his team try to figure out what each one does.

“We don’t have the slightest idea what two-thirds of the things insects are producing are or what they do,” says Ribeiro. “It’s like having a book in front of us that we can read but not understand.”

Not knowing what bugs know has only increased Ribeiro’s respect for the often-maligned creatures. “Yes, bugs are a problem, they transmit disease, and they’re annoying,” he says. “But they also represent incredible knowledge.” Hey, someone has to bite into it.

—Rebecca A. Clay
Bacterial Growth Activity

READ FIRST!
*Pseudomonas fluorescens*, the bacteria used in this activity, does not cause disease in healthy people. Notify your teacher if you have a weakened immune system (which could be due to antibiotic therapy, treatment with immunosuppressive drugs or cancer drugs, AIDS, being HIV-positive, or any other reason) so that an alternative experience that is safer can be provided.

DIRECTIONS: Test the hypothesis using *Pseudomonas fluorescens* and the antibiotic kanamycin. The flow chart provides an overview of the activity.

DAY 1

Materials

- 1 test tube culture of *P. fluorescens* (parental culture)
- 1 test tube containing nutrient broth
- 1 test tube containing nutrient broth with kanamycin (10 mg/mL)
- 1 nutrient agar plate
- 1 nutrient agar plate with kanamycin
- 4 sterile 1-mL pipettes
- 1 pipette pump or bulb
- 1 container with disinfectant for used pipettes
- Bunsen burner
- 1 grease pencil
- 1 beaker of alcohol
- 1 bent glass rod spreader

Aseptic techniques are required for a safe and successful activity. Discard used cultures safely. Your teacher will explain and demonstrate aseptic techniques and indicate where you should discard used cultures (with caps and lids in place). Your teacher will decontaminate all the cultures before disposal.

1. Swirl the *P. fluorescens* culture gently to distribute the bacterial cells evenly. Follow your teacher’s instructions for sterile conditions to transfer 0.1 mL from the culture into the test tube of nutrient broth and into the test tube of nutrient broth with kanamycin. Label the first test tube “A” and the second “B.”

2. Swirl the culture again. Follow your teacher’s instructions to deposit 0.1 mL from the culture on each nutrient agar plate. Spread the culture evenly over the plate’s surface with a sterile bent glass rod. Label the nutrient agar plate “1” and the nutrient agar plate with kanamycin “2.”

3. Allow the culture to soak into the plates for 5 to 10 minutes. Then, invert and incubate them and the 2 broth cultures at 25°C for 3 days.

DAY 2 (3 days later)

4. Retrieve Cultures A and B. Collect 2 new nutrient agar plates and 2 nutrient agar plates with kanamycin.

5. Swirl Culture A gently and follow Step 3 to prepare 2 plates: 1 nutrient agar plate and 1 nutrient agar plate with kanamycin. Label the first plate “3” and the second plate “4.”

6. Swirl Culture B gently and repeat Step 3 using samples from this culture. Label the nutrient agar plate “5” and the nutrient agar plate with kanamycin “6.”

7. Repeat Step 3. Dispose of the A and B cultures as your teacher directs.

DAY 3 (2 to 3 days later)

8. Draw the amount of bacterial growth on each plate on the flow chart.

Discussion Questions

Answer the following questions based on your results.

1. Compare the bacterial growth on Plates 1 and 2, the parental culture. Which has more growth? Explain. Explain the presence of bacteria on the plate containing kanamycin.

2. Compare the growth on Plates 3 and 4, prepared from Culture A (without kanamycin). How does the growth on the plates with and without kanamycin appear? What does this tell you about the bacterial growth in Culture A?

3. Compare the growth on Plates 5 and 6, prepared from Culture B (with kanamycin). How does the growth on the plate with and without kanamycin appear? What does this tell you about the bacterial growth in Culture B?

4. Compare the growth of Cultures A and B on Plates 4 and 6 (with kanamycin). Explain how Culture B could have so many more resistant bacteria than Culture A, even though they both came from the same parental culture.

5. How do you explain the presence of some resistant bacteria in the parental culture and Culture A?

Adapted with permission from BSCS for the publication *The NIH Supplement: Emerging and Reemerging Diseases* (1999).
GLOSSARY

Epidemiology: Study of the cause and course of disease.
Antimicrobials: Agents that kill or stop the progression of bacteria, viruses, and parasites.
Quarantine: Keeping people exposed to an illness away from other people.
Pathogen: Organism that can cause disease in its host.
Vaccine: Substance administered to produce or increase immunity to a particular pathogen.
Vector: Organism that transfers a pathogen from one host to another.
Zoonotic: Pathogen or disease that is transmitted from an animal to a person.

RESOURCES

Ethics—Dark Winter
<homelandsecurity.org/darkwinter/index.cfm>
<cato.org/pubs/pas/pa434.pdf>

Superbugs

Infectious Diseases
Avian Flu—World Health Organization
<who.int/csr/disease/avian_influenza/en/>

“Epidemic!: The World of Infectious Disease” (Disease Transmission)
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