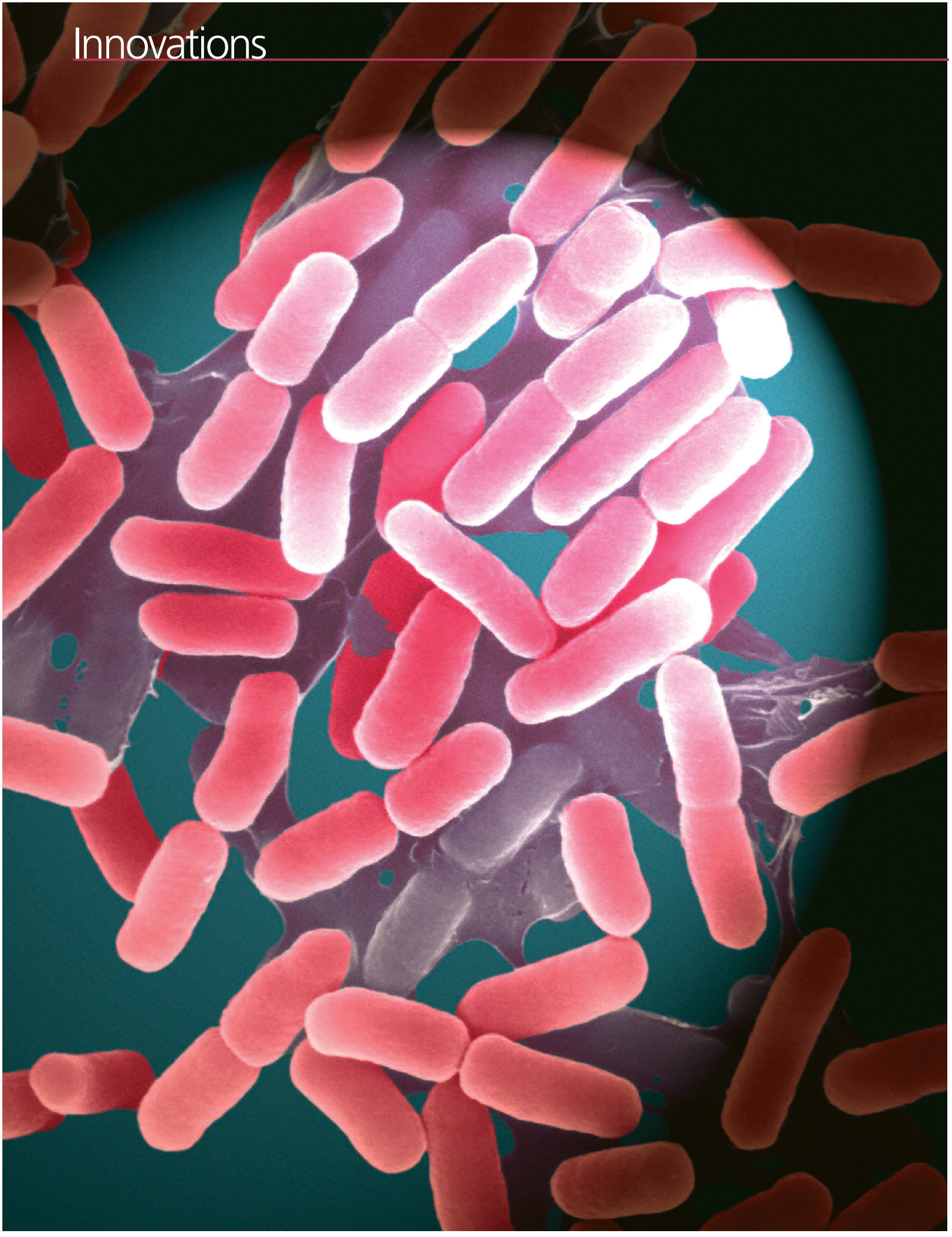
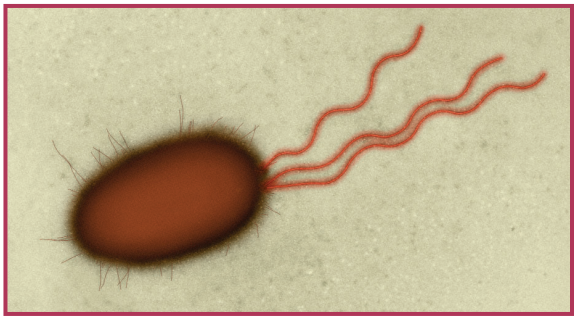


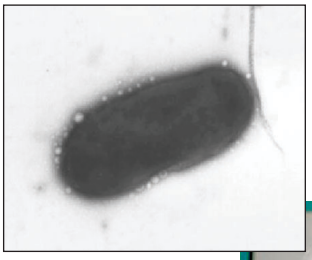
E. coli EXPOSED!

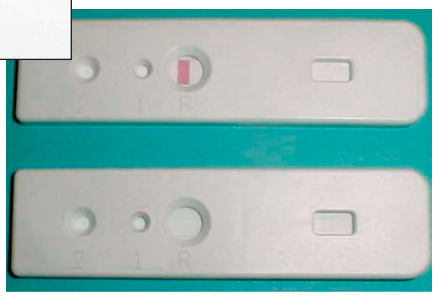
Recent decades have seen new microbial pathogens emerge to threaten human health, especially the health of children. With their lower body weight and immature immune systems, children are more vulnerable than adults to foodborne illnesses caused by the likes of *Campylobacter*, *Salmonella*, *Listeria monocytogenes,* and *Escherichia coli* O157:H7. In the battle to identify contaminated food and the sources of these pathogens, many research efforts have focused on *E. coli* O157:H7, which causes some of the most severe symptoms of foodborne illness in children and has been identified as the source of several major disease outbreaks in the United States.



This page: Dennis Kunkel Microscopy, Inc. Facing page: Dennis Kunkel Microscopy, Inc.; Matt Ray/EHP

As evidence of the dangers of *E. coli* O157:H7 has mounted, the search for technologies to rapidly detect and quantify the bacterium has also intensified. As few as 10 *E. coli* O157:H7 bacteria can cause illness, so sensitivity to low numbers or the ability to amplify cell numbers quickly to detectable levels are highly desirable traits of any testing method. Before testing can be done, most current clinical test methods require an enrichment period of at least 8 hours, during which the sample is placed in a nutritional culture medium or environment so that small numbers of the cells will multiply to a detectable quantity. But separate efforts by researchers at Cornell University and the U.S. Department of Agriculture are building on existing molecular technologies to improve test speed and sensitivity, and produce a simple, potentially low-cost field screening device.





Gotcha! Immunoliposomes tag and identify *E. coli* (left), while test strips show positive and negative results (right).

Long Existence, Short History

E. coli is a normal inhabitant of the intestines of all animals, including humans. It serves a useful function in the body by suppressing the growth of harmful bacteria and by synthesizing vitamins. The Centers for Disease Control and Prevention (CDC) discovered the subgroup, or serotype, *E. coli* O157:H7 in 1975, but it wasn't until 1982 that it was conclusively linked to enteric diseases. Outbreaks of *E. coli* O157:H7–related disease have been recorded worldwide.

E. coli O157:H7 achieved notoriety in 1993, when undercooked contaminated hamburger from the Jack In The Box restaurant chain caused over 700 people, primarily children, to become ill and 4 people to die. Since then, the safety of ground beef has been a concern to health officials, the food industry, and consumers. This past summer, for example, ConAgra Foods recalled 19 million pounds of potentially contaminated ground beef after an *E. coli* O157:H7 outbreak affected more than 35 people. The CDC estimates that the overall number of people sickened in the United States each year by *E. coli* O157:H7 is approximately 73,000, although the incidence of reported cases has decreased somewhat.

A primary source of *E. coli* O157:H7 is the gastrointestinal tract of cattle. The bacterium is transmitted to humans in part by bovine feces, which can taint water used to irrigate crops and can also contaminate meat through improper food handling during processing. *E. coli* O157:H7 outbreaks or sporadic cases have been linked to drinking water, salad bar items, salami, yogurt, and apple juice and cider, the latter of which may have been contaminated when apples on the ground came in contact with manure. In addition, children have become infected after swimming in contaminated water. In 1998, an *E. coli* O157:H7 outbreak at an Atlanta, Georgia, water park hospitalized several children.

Although many cases of foodborne illnesses are acute, they tend to last a short time, and only a few result in extended illnesses. With *E. coli* O157:H7, the incubation period is usually 3–5 days. The bacterium produces toxins that result in abdominal cramps, vomiting, often bloody diarrhea, and sometimes fever. In some acute cases, the infection will develop into hemolytic uremic syndrome (HUS), which is characterized by the destruction of red blood cells, kidney failure, and potentially seizures, stroke, and death. In the elderly, HUS can combine with fever and other symptoms to produce the HUS-like blood disorder thrombotic thrombocytopenic purpura, with a mortality rate at high as 50%.

Children—notably those younger than 4 or 5 years—are at relatively high risk for infection, with *E. coli* O157:H7 possibly the leading cause of acute kidney failure and HUS in young children and infants. According to the CDC, the infection rate for *E. coli* O157:H7 is 6.1 per 100,000 infants and 8.2 per 100,000 children aged 1–9 years. This is the highest infection rate of any age group.

Micro-Approach to a Big Problem

At Cornell University's Food Research Laboratory in Geneva, New York, chemistry professor Richard Durst has been working on using liposomes to deliver markers that reveal the presence of *E. coli* O157:H7 and other pathogens in food samples. Liposomes are spherical nanoscale structures composed of a two-layer phospholipid/cholesterol shell that can encapsulate a variety of different markers, from fluorescent dyes to electroactive compounds. Because the shell of the liposome consists of chemically reactive elements, various surface tags can be attached, including antibodies, antigens, oligonucleotide probes, and cell receptors.

Durst says, "With so much flexibility in what we can attach or encapsulate, there are numerous different formats we can use. We've developed formats in the form of dipsticks similar to home pregnancy tests, optically based lateral-flow systems, fluorescence-based tests, and microfluidic devices. As long as you have an antibody, gene probe, or natural receptor, you can make a bioanalytic sensor with this [liposome] technology."

In a simple paper strip test, antibodies are attached to the outer shell of the liposome and bind to *E. coli* O157:H7 in the sample when mixed together. The liposomes contain dye molecules that clearly mark the pathogens—as the sample liquid is drawn up the paper strip, the paper turns red if *E. coli* O157:H7 is present. The quantity of bacteria present is then estimated by optically measuring the color intensity, which is proportional to the pathogen population.

The microcassette-size device can detect large numbers of *E. coli* O157:H7 in under 10 minutes, although several hours are required for single-pathogen detection. Existing technologies can also quickly identify the bacteria, but only after an 8-hour culture amplification time to produce a detectable count of 10^5 or 10^6 bacteria. "Because each liposome contains hundreds of thousands to perhaps millions of marker molecules, there is a large amplification effect," says Durst. "Since detection and signal amplification using liposome labels are not dependent on a secondary reaction—as required in conventional enzyme-based tests—the use of liposomes can provide almost immediate warning of the presence of a pathogen."

The technology has been investigated for detecting chemical and biological warfare agents, naturally occurring pathogens such as the parasite *Cryptosporidium parvum*, viruses such as herpes, and natural toxins such as botulinum. Independent field testing of the device, sponsored by the New York State Energy Research and Development Authority, is now under way

Cornell University Food Research Laboratory

through a county public health laboratory.

Antje Baeumner, a former postdoctoral fellow of Durst's who is now an assistant professor in the Department of Biological and Environmental Engineering at Cornell, is developing a complementary detection technology in which liposomes are used in conjunction with a microfluidics-based biosensing system. The technology of microfluidics encompasses devices and processes that deal with very low quantities—usually nanoliters—of fluids. This scale changes the behavior of systems, including the capillary action by which fluids pass through channels. So with very small channels, liquid can be controlled in new and useful ways. Baeumner's goal in this case is to create a micro–Total Analysis System (μ-TAS), a "lab-on-a-chip" containing automated microchannels that perform all the steps necessary, including sample preparation, amplification, and electrochemical detection and quantification of pathogens—all on a device the size of a handheld computer.

In one of the μ-TAS component chips, she uses liposomes encapsulating an electroactive couple (an oxidized and reduced form of the same molecule). This molecule is used instead of a visible marker to indicate targets such as *E. coli* O157:H7. In a chamber on the chip, nanoscale cathode and anode pads with interlocking fingers alternately oxidize and then reduce the couple repeatedly. As this oxidation–reduction reaction continues, the resulting electron flow through the microelectrodes can be easily monitored, and the magnitude of the signal is directly proportional to the concentration of *E. coli* O157:H7 present.

The technologies under development by Durst and Baeumner are being evaluated by several large companies and government agencies. The licensing arrangements, field trials, and potential commercialization of the technologies are handled through the offices of Innovative Biotechnologies International. Richard Montagna, the company's president, says a commercial biosensor for *C. parvum* should be available in early 2003, while one for *E. coli* O157:H7 is about a year away.

The Shape of Assays to Come

Other weapons against *E. coli* O157:H7 are in earlier phases of development. For example, at the U.S. Department of Agriculture's Animal Waste Pathogen Laboratory in Beltsville, Maryland, microbiologist Daniel Shelton is working on a method to quickly identify and quantify *E. coli* O157:H7 in both natural and constructed bodies of water.

Shelton is using magnetic beads coated with anti–*E. coli* O157:H7 monoclonal antibodies to bind to the bacterium. He is not ready to reveal the specific culture medium, but he says it provides a very fast means of growing detectable levels of the bacterium—a day or less—whereas traditional water testing can take up to four days. This method will allow identification of 1 *E. coli* O157:H7 bacterium in 100 milliliters of water, and should help health officials check public water sites such as swimming pools.

Shelton says magnetic bead technology is not new—commercially available immunoassay kits already use magnetic beads to deliver chemical markers for all different purposes. But Shelton's method is unique in the rapidity with which the user will be able to quantify *E. coli* O157:H7 in a sample. Plus, most other research efforts have focused on detecting the contaminant in food, rather than water. None of Shelton's research has been published yet, and he is still waiting on results from current studies, but the water treatment industry and public water works agencies have shown interest in his work.

However, acceptance of these new techniques will come only if they prove themselves over time, according to Michael Doyle, a professor of food microbiology and head of the Center for Food Safety at the University of Georgia, who has conducted his own developmental work on detecting *E. coli* O157:H7. Doyle notes that the biggest and an often overlooked challenge in developing any fast detection method is the fact that a large portion of the *E. coli* O157:H7 population in any sample is often damaged by heat, freezing, or chlorine in the water.

Doyle says the only way to ensure that all *E. coli* O157:H7 are counted is to give them a long enough incubation period to allow them to revive, which is what they would do inside the human body. Durst agrees, but adds that his method is based on high sensitivity to low numbers of organisms and will quickly detect, though perhaps not accurately quantify, *E. coli* O157:H7 even if large numbers of the organism are dormant. He says, "Our liposome immunoassay detection limit without enrichment is about one thousand target organisms per milliliter or gram. Most immunobased assays require the presence of at least ten to one hundred thousand cells of the target organism per milliliter or gram for detection. Consequently, with this ten- to one hundred–fold increase in sensitivity, we require only about a three-hour enrichment to achieve a less-than-one-target-organism-per-milliliter or -gram detection."

The food industry would like to make use of these technologies and applauds their development for testing both food and water. Yet there is more that should be done. Jenny Scott, senior director of Food Safety Programs at the National Food Processors Association, a Washington, D.C.–based trade association, says that testing is only part of the answer to the threat posed by *E. coli* O157:H7. "There are limitations with any testing method," she says. "No matter how good, it's never good enough."

According to Scott, the entire food chain from farm to kitchen must be addressed, with consumers educated about the need to cook food long enough to kill bacteria and the potential value of food irradiation. Other key steps are to properly handle and test food during processing, and to start with good farm practices and science, such as controlling manure properly and possibly developing a vaccine for *E. coli* O157:H7 in cattle.

W. Conard Holton

Suggested Reading

Buzby JC. 2001. Children and microbial foodborne illness. FoodReview 24(2):32–37.

Feng P. 1995. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. Emerg Infect Dis 1(2):47–52.

IFT. 2000. Emerging Microbiological Food Safety Issues: Implications for Control in the 21st Century. Chicago, IL: Institute of Food Technologists.

Park S, Worobo RW, Durst RA. 1999. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: a literature review. Crit Rev Food Sci Nutr 39(6):481–502.