Mom may have warned you not to eat food from a can that’s dented, swollen, or leaking. As usual, she was right. Her concern may have been that you’d get botulism.

This illness is caused by botulinum toxin, which is produced by a soil-dwelling microbe, Clostridium botulinum, and several of its Clostridium cousins. A damaged can could become contaminated, and, in the dark and warmth of your kitchen cupboard, the microbe could flourish—producing the deadly toxin.

Even though cases of foodborne botulism poisoning are rare in the United States today, they’re nonetheless of concern. After all, botulinum toxin is the world’s most potent natural toxin—millions of times more poisonous than cyanide.

The toxin and the Clostridium microbes that produce it are the focus of studies by ARS food safety experts at the Foodborne Contaminants Research Unit, part of the agency’s Western Regional Research Center in Albany, California, near San Francisco. These scientists are developing new and better ways to detect the toxin quickly, easily, and inexpensively. And they’re looking for important differences in the potency of the natural toxin—the way it occurs in nature—as compared to purified toxin, the kind most scientists prefer to study.

**Portrait of a Killer**

Botulinum toxin occurs in seven different forms—serotypes A through G. Serotypes A and B are culprits in about 90 percent of the foodborne botulism cases in the United States. Within this suite of seven serotypes, scientists have identified more than 100 subtypes.

No one knows for certain why *C. botulinum* produces the toxins. “It probably produces toxins to kill the organism that it is invading,” says John Mark Carter, chemist and leader of the Albany research team.

Botulinum is a protein that behaves as a neurotoxin, interfering with the neurological system that would otherwise transmit vital signals throughout your body. In particular, the toxin can cut off normal messaging to muscles, causing paralysis. In the worst case, the toxin paralyzes the muscles of the diaphragm, leading to death by suffocation.

There’s no federally approved vaccine against botulinum. An injection of horse antiserum can help remove the toxin from a patient’s bloodstream, but this treatment may produce serious side effects. Victims who don’t die from botulism poisoning require months of treatment.

According to the U.S. Centers for Disease Control and Prevention, botulism symptoms include drooping eyelids, slurred speech, blurred or double vision, dry mouth, and, of course, weakening and paralysis of muscles. The good news is that, in most instances, botulinum toxin in food can be inactivated by routine cooking—boiling, baking, or roasting, for example.

**New Antibodies Improve Detection**

For decades, the gold standard of tests for detecting botulinum toxin has been an assay that requires the use of laboratory mice. The assay takes at least 4 days, preferably 8, to perform correctly and is neither portable nor economical. That’s why biologist Larry Stanker and two Albany colleagues—biologist Luisa Cheng and biochemist Miles Scotcher—developed a new and improved assay for detecting botulinum toxin serotype A.
The new assay is based on laboratory-built molecules, known as “monoclonal antibodies,” which can bind to the toxin. Monoclonal antibodies that bind to serotype A toxin aren’t new, but the ones the Albany team developed may be the most sensitive yet produced, that is, they’re capable of detecting the toxin in minuscule amounts.

Stanker has formatted these antibodies into an assay that’s 10 times more sensitive than the mouse assay, yet is less expensive and is easier to use.

Stanker expects to complete assays for detecting serotypes B and E sometime this year. Already, he has worked with Safeguard Biosystems, Inc., of San Diego, California, to package two of the serotype A antibodies into a dipstick-style test kit that looks and operates much like a home pregnancy test. The botulinum kit is intended for testing liquids, such as beverages, or clinical specimens such as blood or urine.

**Test Tells Toxin’s Activity Level**

Though it has many advantages over the mouse assay, the dipstick test can’t differentiate activated from inactivated toxin. To address this problem, chemist Reuven Rasooly and Albany colleagues incorporated one of the new serotype A antibodies into a test that determines whether the toxin is active or inactive.

Toxin that’s been inactivated by heating “won’t show up in this test,” Rasooly says.

The test sequentially targets two different parts, or subunits—a so-called “heavy chain” and a “light chain”—of the botulinum toxin. For the test, magnetic plastic beads—about 5,000 times smaller than a marble—are coated with one of the new antibodies. Then, the antibody-coated beads are exposed to a sample, such as a fruit juice.

If there are any botulinum toxin molecules in the juice, their heavy-chain subunits will bind to the monoclonal antibody on the magnetic beads. This isolates and concentrates the toxin on the beads, allowing it to be easily extracted from the sample by collecting the beads with a magnet.

Next, these toxin-coated beads are exposed to a liquid containing laboratory-built molecules known as “synthetic peptides.” The light-chain subunits of active toxin can cut these peptides neatly in two.

In the human body, this cleaving disrupts neuron function and leads to muscular paralysis. In the laboratory test, this same cleaving causes the peptides to fluoresce. Only active toxin—not heat-inactivated toxin—can cut the peptides. Thus, the fluorescence—which can be measured easily and with high sensitivity—is an excellent indication of the activity level of the toxin.

The idea of measuring the cleaving of peptides isn’t new, nor are the magnetic beads. It’s Rasooly’s particular combination of the magnetic beads, the synthetic fluorescing peptide, and the superior new monoclonal antibody that make his peptide-
Using protein gel electrophoresis, Luisa Cheng separates crude botulinum toxin into individual protein bands that will help identify which protein component could affect toxicity.

The botulinum toxin assay is comparatively inexpensive and takes only about 20 minutes to perform.

Besides investigating botulinum toxin, chemist Reuven Rasooly is studying toxins produced by Staphylococcus aureus. Here, after extracting staph toxin from food, Rasooly and bioscience technician Paula Do look for replication of spleen cells. Replication is a measure of the amount of biologically active toxin.